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(54) Title: 33521, A NOVEL HUMAN GUANINE NUCLEOTIDE EXCHANGE FAMILY MEMBER AND USES THEREOF

(57) Abstract: The invention provides isolated nucleic acids molecules, designated 33521 nucleic acid molecules, which encode novel Rho GEF members. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing 33521 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a 33521 gene has been introduced or disrupted. The invention still further provides isolated 33521 proteins, fusion proteins, antigenic peptides and anti-33521 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

**33521, A NOVEL HUMAN GUANINE NUCLEOTIDE EXCHANGE
FAMILY MEMBER AND USES THEREOF**

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Related Applications

This application claims priority to U.S. provisional application number 60/235,033 filed on September 25, 2000, the content of which is incorporated herein by reference.

Background of the Invention

Rho small GTPases are critical cellular regulators. Rho proteins regulate the formation of actin structures, particularly stress fibers and focal adhesions. For example, the Rho family member, Rac regulates membrane ruffling and lamellipodia formation in fibroblasts. Cdc42, another family member, regulates filopodia formation. Rho proteins also have more disparate roles in cell cycle progression, gene transcription through the serum response factor, Ras-mediated transformation, and NADPH oxidase-mediated phagocytosis (see Fleming *et al.* (1999) *J Biol Chem* 274:12753 for a review). Rho proteins, like other small GTPases, cycle between two different conformations: one bound to GTP and one to GDP. The different conformations have different regulatory properties (see, e.g., Park *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94:4463-8.) The GTP bound protein can hydrolyze GTP to convert to the GDP-bound form. This event is stimulated by a GTPase-activating protein (GAP). Likewise, the GDP-bound form can convert to the GTP bound form by releasing the bound GDP and exchanging it for GTP. This step is stimulated by a GDP exchange factor (GEF). Thus, GAPs and GEFs are key regulators in controlling Rho-mediated signalling events.

One particular class of Rho GEFs is the TIAM class which have the hallmark domain organization of – from amino- to carboxy-terminus – a first plekstrin homology (PH) domain, a Raf-like Ras binding domain (RBD), a PDZ domain, a Rho GEF domain, and a second PH domain. The PH, PDZ, and RBD domains are common small modules of signalling proteins. PH domains, as described below, can potentially interact with lipids, membranes, and inositol signalling molecules. PDZ domain are peptide binding domains, that frequently recognize the carboxy-termini of proteins. RBD domains are involved in signalling by Ras, a key mediator of cell proliferation and oncogenesis.

The TIAM class of GEFs include TIAM1, TIAM2, Still-life protein type 1 (SIF type 1), and Still-life protein type 2 (SIF type 2). TIAM1 was originally identified as a T-lymphoma invasion and metastasis inducing protein. TIAM1 can induce the invasion of T-lymphoma cells into a fibroblast monolayer by activating Rac1, a Rho class GTPase.

5 Activation of Rac1 activity can modulate the assembly of adherens junctions, and cell adhesion, e.g., E-cadherin-mediated adhesion (Hordijk *et al.* (1997) *Science* 278:1464-1465; Habets *et al.* (1994) *Cell* 77:537; Fischels *et al.* (1995) *Nature* 375:338). TIAM1 can be stimulated by a variety of signals. For example, hyaluronic acid binding to CD44 results in a CD44 interaction with TIAM1 that increases Rac1 activity (Bourguignon (2000) *J Biol Chem* 10 275:1829). Thus, extracellular environment and signals can regulate cell motility and morphogenesis through the TIAM1 protein. The *Drosophila* TIAM1 homolog, SIF, is similarly involved in controlling cell morphogenesis, particular the morphology of synaptic terminals (Sone *et al.* (1997) *Science* 275:543).

In sum, the TIAM1 class of GEFs are key intracellular signalling molecules which 15 have multiple protein domains, including a GEF domain that regulates Rho GTPases. These proteins are likely to be critical in a variety of physiological systems, including, but not limited to immune cells, epithelial cells, and neurons as well as pathological systems such as tumor cells and metastatic cells.

Summary of the Invention

20 The present invention is based, in part, on the discovery of a novel Rho GDP exchange factor (Rho GEF) family member, referred to herein as "33521". The nucleotide sequence of a cDNA encoding 33521 is shown in SEQ ID NO:1, and the amino acid sequence of a 33521 polypeptide is shown in SEQ ID NO:2. In addition, the nucleotide sequences of the coding region are depicted in SEQ ID NO:3.

25 Accordingly, in one aspect, the invention features a nucleic acid molecule that encodes a 33521 protein or polypeptide, e.g., a biologically active portion of the 33521 protein. In a preferred embodiment the isolated nucleic acid molecule encodes a polypeptide having the amino acid sequence of SEQ ID NO:2. In other embodiments, the invention provides isolated 33521 nucleic acid molecules having the nucleotide sequence shown in 30 SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number _____. In still other embodiments, the invention provides

nucleic acid molecules that are substantially identical (e.g., naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number _____. In other embodiments, the invention provides a nucleic acid molecule which hybridizes under a 5 stringency condition described herein to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number _____, wherein the nucleic acid encodes a full length 33521 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs that include 10 a 33521 nucleic acid molecule described herein. In certain embodiments, the nucleic acid molecules of the invention are operatively linked to native or heterologous regulatory sequences. Also included, are vectors and host cells containing the 33521 nucleic acid molecules of the invention e.g., vectors and host cells suitable for producing 33521 nucleic acid molecules and polypeptides.

15 In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of 33521-encoding nucleic acids.

In still another related aspect, isolated nucleic acid molecules that are antisense to a 33521 encoding nucleic acid molecule are provided.

20 In another aspect, the invention features, 33521 polypeptides, and biologically active or antigenic fragments thereof that are useful, e.g., as reagents or targets in assays applicable to treatment and diagnosis of 33521-mediated or -related disorders. In another embodiment, the invention provides 33521 polypeptides having a 33521 activity. Preferred polypeptides are 33521 proteins including at least one Rho GEF domain; at least one, preferably two PH domains; at least one PDZ domain; and, preferably, having a 33521 activity, e.g., a 33521 25 activity as described herein.

In other embodiments, the invention provides 33521 polypeptides, e.g., a 33521 30 polypeptide having the amino acid sequence shown in SEQ ID NO:2 or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC Accession Number _____; an amino acid sequence that is substantially identical to the amino acid sequence shown in SEQ ID NO:2 or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC Accession Number _____; or an amino acid sequence

encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under a stringency condition described herein to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number _____, wherein the nucleic acid encodes a full length 33521 protein or an active fragment thereof.

5 In a related aspect, the invention further provides nucleic acid constructs which include a 33521 nucleic acid molecule described herein.

In a related aspect, the invention provides 33521 polypeptides or fragments operatively linked to non-33521 polypeptides to form fusion proteins.

10 In another aspect, the invention features antibodies and antigen-binding fragments thereof, that react with, or more preferably specifically bind 33521 polypeptides or fragments thereof.

In another aspect, the invention provides methods of screening for compounds that modulate the expression or activity of the 33521 polypeptides or nucleic acids.

15 In still another aspect, the invention provides a process for modulating 33521 polypeptide or nucleic acid expression or activity, e.g. using the screened compounds. In certain embodiments, the methods involve treatment of conditions related to aberrant activity or expression of the 33521 polypeptides or nucleic acids, such as conditions involving aberrant or deficient cellular proliferation or differentiation.

20 The invention also provides assays for determining the activity of or the presence or absence of 33521 polypeptides or nucleic acid molecules in a biological sample, including for disease diagnosis.

In yet another aspect, the invention provides methods for inhibiting the proliferation or inducing the killing, of a 33521-expressing cell, e.g., a hyper-proliferative 33521-expressing cell. The method includes contacting the cell with a compound (e.g., a compound identified using the methods described herein) that modulates the activity, or expression, of the 33521 polypeptide or nucleic acid. In a preferred embodiment, the contacting step is effective *in vitro* or *ex vivo*. In other embodiments, the contacting step is effected *in vivo*, e.g., in a subject (e.g., a mammal, e.g., a human), as part of a therapeutic or prophylactic protocol. In a preferred embodiment, the cell is a hyperproliferative cell, e.g., a cell found in a solid tumor, a soft tissue tumor, or a metastatic lesion.

In a preferred embodiment, the compound is an inhibitor of a 33521 polypeptide. Preferably, the inhibitor is chosen from a peptide, a phosphopeptide, a small organic molecule, a small inorganic molecule and an antibody (e.g., an antibody conjugated to a therapeutic moiety selected from a cytotoxin, a cytotoxic agent and a radioactive metal ion).

5 In another preferred embodiment, the compound is an inhibitor of a 33521 nucleic acid, e.g., an antisense, a ribozyme, or a triple helix molecule.

In a preferred embodiment, the compound is administered in combination with a cytotoxic agent. Examples of cytotoxic agents include anti-microtubule agent, a topoisomerase I inhibitor, a topoisomerase II inhibitor, an anti-metabolite, a mitotic inhibitor, 10 an alkylating agent, an intercalating agent, an agent capable of interfering with a signal transduction pathway, an agent that promotes apoptosis or necrosis, and radiation.

In another aspect, the invention features methods for treating or preventing a disorder characterized by aberrant cellular proliferation or differentiation of a 33521-expressing cell, in a subject. Preferably, the method includes administering to the subject (e.g., a mammal, 15 e.g., a human) an effective amount of a compound (e.g., a compound identified using the methods described herein) that modulates the activity, or expression, of the 33521 polypeptide or nucleic acid. In a preferred embodiment, the disorder is a cancerous or pre-cancerous condition.

In a further aspect, the invention provides methods for evaluating the efficacy of a 20 treatment of a disorder, e.g., proliferative disorder or a disorder. The method includes: treating a subject, e.g., a patient or an animal, with a protocol under evaluation (e.g., treating a subject with one or more of: chemotherapy, radiation, and/or a compound identified using the methods described herein); and evaluating the expression of a 33521 nucleic acid or polypeptide before and after treatment. A change, e.g., a decrease or increase, in the level of 25 a 33521 nucleic acid (e.g., mRNA) or polypeptide after treatment, relative to the level of expression before treatment, is indicative of the efficacy of the treatment of the disorder. The level of 33521 nucleic acid or polypeptide expression can be detected by any method described herein.

In a preferred embodiment, the evaluating step includes obtaining a sample (e.g., a 30 tissue sample, e.g., a biopsy, or a fluid sample) from the subject, before and after treatment

and comparing the level of expressing of a 33521 nucleic acid (e.g., mRNA) or polypeptide before and after treatment.

In another aspect, the invention provides methods for evaluating the efficacy of a therapeutic or prophylactic agent (e.g., an anti-neoplastic agent). The method includes:

- 5 contacting a sample with an agent (e.g., a compound identified using the methods described herein, a cytotoxic agent) and, evaluating the expression of 33521 nucleic acid or polypeptide in the sample before and after the contacting step. A change, e.g., a decrease or increase, in the level of 33521 nucleic acid (e.g., mRNA) or polypeptide in the sample obtained after the contacting step, relative to the level of expression in the sample before the contacting step, is indicative of the efficacy of the agent. The level of 33521 nucleic acid or polypeptide expression can be detected by any method described herein. In a preferred embodiment, the sample includes cells obtained from a cancerous tissue.
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In further aspect, the invention provides assays for determining the presence or absence of a genetic alteration in a 33521 polypeptide or nucleic acid molecule, including for disease diagnosis.

In another aspect, the invention features a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., a nucleic acid or peptide sequence. At least one address of the plurality has a capture probe that recognizes a 33521 molecule. In one embodiment, the capture probe is a nucleic acid, e.g., a probe complementary to a 33521 nucleic acid sequence. In another embodiment, the capture probe is a polypeptide, e.g., an antibody specific for 33521 polypeptides. Also featured is a method of analyzing a sample by contacting the sample to the aforementioned array and detecting binding of the sample to the array.

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Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

Figure 1 depicts a hydropathy plot of human 33521. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence of

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human 33521 are indicated. Polypeptides of the invention include fragments which include:
all or part of a hydrophobic sequence, i.e., a sequence above the dashed line, e.g., the
sequence from about amino acid 722 to 730, from about 883 to 891, and from about 966 to
975, of SEQ ID NO:2; all or part of a hydrophilic sequence, i.e., a sequence below the dashed
line, e.g., the sequence of from about amino acid 741 to 750, from about 756 to 762, and
from about 1363 to 1372, of SEQ ID NO:2; a sequence which includes a Cys, or a
glycosylation site.

Figure 2A depicts an alignment of the first PH domain of human 33521 with a
consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM.
The upper sequence is the consensus amino acid sequence (SEQ ID NO:4), while the lower
amino acid sequence corresponds to amino acids 507 to 620 of SEQ ID NO:2.

Figure 2B depicts an alignment of the RBD domain of human 33521 with a consensus
amino acid sequence derived from a hidden Markov model (HMM) from PFAM. The upper
sequence is the consensus amino acid sequence (SEQ ID NO:5), while the lower amino acid
sequence corresponds to amino acids 810 to 853 of SEQ ID NO:2.

Figure 2C depicts an alignment of the PDZ domain of human 33521 with a consensus
amino acid sequence derived from a hidden Markov model (HMM) from PFAM. The upper
sequence is the consensus amino acid sequence (SEQ ID NO:6), while the lower amino acid
sequence corresponds to amino acids 890 to 975 of SEQ ID NO:2.

Figure 2D depicts an alignment of the Rho GEF domain of human 33521 with a
consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM.
The upper sequence is the consensus amino acid sequence (SEQ ID NO:7), while the lower
amino acid sequence corresponds to amino acids 1103 to 1292 of SEQ ID NO:2.

Figure 2E depicts an alignment of the second PH domain of human 33521 with a
consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM.
The upper sequence is the consensus amino acid sequence (SEQ ID NO:8), while the lower
amino acid sequence corresponds to amino acids 1353 to 1455 of SEQ ID NO:2.

Figure 3A depicts an alignment of the first PH domain of human 33521 with a
consensus amino acid sequence derived from a hidden Markov model (HMM) from SMART.
The upper sequence is the consensus amino acid sequence (SEQ ID NO:9), while the lower
amino acid sequence corresponds to amino acids 507 to 622 of SEQ ID NO:2.

Figure 3B depicts an alignment of the RBD domain of human 33521 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from SMART. The upper sequence is the consensus amino acid sequence (SEQ ID NO:10), while the lower amino acid sequence corresponds to amino acids 810 to 881 of SEQ ID NO:2.

5 *Figure 3C* depicts an alignment of the PDZ domain of human 33521 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from SMART. The upper sequence is the consensus amino acid sequence (SEQ ID NO:11), while the lower amino acid sequence corresponds to amino acids 900 to 976 of SEQ ID NO:2.

10 *Figure 3D* depicts an alignment of the Rho GEF domain of human 33521 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from SMART. The upper sequence is the consensus amino acid sequence (SEQ ID NO:12), while the lower amino acid sequence corresponds to amino acids 1103 to 1292 of SEQ ID NO:2.

15 *Figure 3E* depicts an alignment of the second PH domain of human 33521 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from SMART. The upper sequence is the consensus amino acid sequence (SEQ ID NO:13), while the lower amino acid sequence corresponds to amino acids 1326 to 1457 of SEQ ID NO:2.

Detailed Description

The human 33521 sequence (see SEQ ID NO:1, as recited in Example 1), which is approximately 5437 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 5106 nucleotides, including the termination codon. The coding sequence encodes a 1701 amino acid protein (see SEQ ID NO:2, as recited in Example 1).

Human 33521 contains the following regions or other structural features:

- two PH (pleckstrin homology) domains (PFAM Accession Number PF00169) located at about amino acid residues 507 to 620 and 1353 to 1455 of SEQ ID NO:2;
- an RBD domain (PFAM Accession Number PF02196) located at about amino acid residues 810 to 881 of SEQ ID NO:2;
- a PDZ domain (PFAM Accession Number PF00595) located at about amino acid residues 890 to 975 of SEQ ID NO:2;
- 30 a Rho guanine nucleotide exchange factor domain (PFAM Accession Number PF00621) located at about amino acid residues 1103 to 1292 of SEQ ID NO:2;

a predicted coiled-coil domain located at about amino acids 629 to 694 of SEQ ID NO:2;

30 predicted protein kinase C phosphorylation sites (PS00005) at about amino acids 29 to 31, 82 to 84, 93 to 95, 129 to 131, 158 to 160, 225 to 227, 237 to 239, 298 to 300, 330 to 332, 370 to 372, 375 to 377, 391 to 393, 440 to 442, 541 to 543, 632 to 634, 755 to 757, 767 to 769, 962 to 964, 1046 to 1048, 1054 to 1056, 1110 to 1112, 1220 to 1222, 1514 to 1316, 1471 to 1473, 1486 to 1488, 1491 to 1493, 1494 to 1496, 1510 to 1512, 1578 to 1580, and 1641 to 1643, of SEQ ID NO:2;

45 predicted casein kinase II phosphorylation sites (PS00006) located at about amino acids 129 to 132, 218 to 221, 229 to 232, 248 to 251, 261 to 264, 298 to 301, 337 to 340, 411 to 414, 471 to 474, 478 to 481, 485 to 488, 491 to 494, 577 to 580, 593 to 596, 604 to 607, 741 to 744, 786 to 789, 797 to 800, 898 to 901, 950 to 953, 984 to 987, 1019 to 1022, 1036 to 1039, 1046 to 1049, 1067 to 1070, 1095 to 1098, 1136 to 1139, 1146 to 1149, 1160 to 1163, 1239 to 1242, 1264 to 1267, 1302 to 1305, 1312 to 1315, 1322 to 1325, 1384 to 1387, 1414 to 1417, 1421 to 1424, 1438 to 1441, 1471 to 1474, 1502 to 1505, 1514 to 1517, 1569 to 1572, 1642 to 1645, 1661 to 1664, and 1672 to 1675, of SEQ ID NO:2;

twelve predicted N-glycosylation sites (PS00001) located at about amino acids 15 to 18, 144 to 147, 291 to 294, 638 to 641, 1003 to 1006, 1069 to 1072, 1131 to 1134, 1382 to 1385, 1412 to 1415, 1500 to 1503, 1640 to 1643, and 1665 to 1668, of SEQ ID NO:2;

six predicted cAMP/cGMP to dependent protein kinase phosphorylation sites (PS00004) located at about amino acids 215 to 218, 372 to 375, 381 to 384, 748 to 751, 781 to 784, and 1016 to 1019, of SEQ ID NO:2;

twenty predicted N-myristylation sites (PS00008) from about amino acids 2 to 7, 49 to 54, 79 to 84, 88 to 93, 105 to 110, 180 to 185, 240 to 245, 315 to 320, 324 to 329, 422 to 427, 763 to 768, 821 to 826, 928 to 933, 934 to 939, 1032 to 1037, 1164 to 1169, 1405 to 1410, 1521 to 1526, 1531 to 1536, and 1618 to 1623, of SEQ ID NO:2;

one predicted glycosaminoglycan site (PS00002) located at about amino acids 1615 to 1618 of SEQ ID NO: 2; and

three predicted tyrosine kinase phosphorylation sites (PS00007) located at about amino acids 401 to 408, 843 to 850, and 856 to 864, of SEQ ID NO: 2.

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer *et al.* (1997) *Protein* 28:405-420 and <http://www.psc.edu/general/software/packages/pfam/pfam.html>.

A plasmid containing the nucleotide sequence encoding human 33521 (clone 5 "Fbh33521FL") was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is 10 not an admission that a deposit is required under 35 U.S.C. §112.

The 33521 protein has the domain organization of the TIAM1 family of proteins. The organization is as follows: a first PH domain, a RBD domain, a PDZ domain, a Rho GEF domain, and a second PH domain. The 33521 protein contains a significant number of structural characteristics in common with members of the Rho GEF family, the PH domain 15 family, and the PDZ domain family. The term "family" when referring to the protein and nucleic acid molecules of the invention means two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a 20 family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologues of non-human origin, e.g., rat or mouse proteins. Members of a family can also have common functional characteristics.

Rho GEF domains are also referred to as Dbl homology domains. The Rho GEF domain typically functions in conjunction with a carboxy-terminal PH domain. The crystal 25 structure of the Rho GEF from the human Son of sevenless (Sos) protein and its adjacent carboxy-terminal PH domain has been determined (Soisson *et al.* (1998) *Cell* 95:259-68). The Rho GEF domain is entirely α -helical with the active site residues positioned near the interface with the PH domain. These two domains may function together in binding the effector region of Rho proteins to thereby trigger GDP release.

30 A 33521 polypeptide can include a "Rho GEF domain" or regions homologous with a "Rho GEF domain".

As used herein, the term "Rho GEF domain" includes an amino acid sequence of about 170 to 190 amino acid residues in length and having a bit score for the alignment of the sequence to the Rho GEF domain (HMM) of at least 145. Preferably, a Rho GEF domain includes at least about 150 to 220 amino acids, more preferably about 160 to 200 amino acid residues, or about 170 to 190 amino acids and has a bit score for the alignment of the sequence to the Rho GEF domain (HMM) of at least 50, 100, preferably 180, or more preferably 200 or greater. The Rho GEF domain (HMM) has been assigned the PFAM Accession Number PF00621 (<http://genome.wustl.edu/Pfam/.html>). An alignment of the Rho GEF domain (amino acids 1103 to 1292 of SEQ ID NO:2) of human 33521 with a PFAM consensus amino acid sequence (SEQ ID NO:7) derived from a hidden Markov model is depicted in Figure 2D. An alignment of the Rho GEF domain (amino acids 1103 to 1292 of SEQ ID NO:2) of human 33521 with a SMART consensus amino acid sequence (SEQ ID NO:12) derived from a hidden Markov model is depicted in Figure 3D.

In a preferred embodiment 33521 polypeptide or protein has a "Rho GEF domain" or a region which includes at least about 150 to 220 more preferably about 160 to 200 or 170 to 190 amino acid residues and has at least about 50%, 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "Rho GEF domain," e.g., the Rho GEF domain of human 33521 (e.g., residues 1103 to 1292 of SEQ ID NO:2).

To identify the presence of a "Rho GEF" domain in a 33521 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against the Pfam database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.* (1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.* (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.* (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference.

A search was performed against the HMM database resulting in the identification of a "Rho GEF" domain in the amino acid sequence of human 33521 at about residues 1103 to 1292 of SEQ ID NO:2 (see Example 1).

PH domains are small domains found in a diverse class of proteins, including those involved in intracellular signaling and the cytoskeleton. The domain has been implicated in binding the $\beta\gamma$ subunit of heterotrimeric G-proteins, lipids (e.g., ascorbyl sterates, and phosphoinositols), and phosphoserine and phosphothreonine. The ligand specificity of PH domains can vary from domain to domain. The structures of multiple PH domains have been determined (for a review, see Riddihough (1994) *Nat. Struct. Biol.* 1:755-757). From analysis of such structures, it is evident that the PH domains represent a conserved fold consisting of two perpendicular β -sheets followed by an amphipathic α -helix despite the lack of absolutely conserved residues. However, the loop regions differ greatly in length and composition. Many Rho GEF domain protein also contain a PH domain. Such proteins included vav, dbl, Sos, yeast CDC24, TIAM1, and Still-life (SIF) proteins.

A 33521 polypeptide can include a "PH domain" or regions homologous with a "PH domain".

As used herein, the term "PH domain" includes an amino acid sequence of about 80 to 120 amino acid residues in length and having a bit score for the alignment of the sequence to the PH domain (HMM) of at least 60. Preferably, a PH domain includes at least about 80 to 130 amino acids, more preferably about 90 to 120 amino acid residues, or about 100 to 115 amino acids and has a bit score for the alignment of the sequence to the PH domain (HMM) of at least 5, 10, 20, 40, 60 or greater. The PH domain (HMM) has been assigned the PFAM Accession Number PF00169 (<http://genome.wustl.edu/Pfam/.html>). Alignments of the PH domains (amino acids 507 to 620 and 1353 to 1455 of SEQ ID NO:2) of human 33521 with a consensus amino acid sequence (SEQ ID NO:4) derived from a PFAM hidden Markov model are depicted in Figure 2A and 2E. Similar alignments with the PH domain consensus from SMART are depicted in Figure 3A and 3E.

In a preferred embodiment 33521 polypeptide or protein has a "PH domain" or a region which includes at least about 80 to 130 more preferably about 90 to 120 or 100 to 115 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "PH domain," e.g., the first PH domain of human 33521 (e.g., residues 507

to 620 of SEQ ID NO:2) or the second PH domain of human 33521 (e.g., residues 1353 to 1455 of SEQ ID NO:2).

To identify the presence of a "PH" domain in a 33521 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of HMMs, e.g., as described above. A "PH" domain was located by such a search in the amino acid sequence of human 33521 at about residues 507 to 620 and 1353 to 1455 of SEQ ID NO:2 (see Example 1).

PDZ domains (also referred to as DHR or GLGF domains) are found in proteins from bacteria to humans. The canonical PDZ domain is a largely β -sheet structure, which has a ligand binding pocket for extended peptides. In eukaryotes, PDZ domain frequently bind to carboxy-terminal peptides, such as the carboxy-terminal tetrapeptide X-[ST]-X-V. The peptide can bind in a groove, and form a β -strand which is antiparallel to a β -strand in the domain, thus, completing an anti-parallel β -sheet. The peptide binding groove can also contain a conserved loop which provides binding pocket for the carboxy-terminus of the ligand. The carboxylate at the terminus hydrogen bonds to main chain amides of the loop, which is characterized by a conserved glycine and a conserved phenylalanine. PDZ domain-containing proteins can mediate protein-protein interactions in signaling complexes, as well as a host of other functions, such as the clustering of ion channels that terminate in PDZ-binding peptides.

A 33521 polypeptide can include a "PDZ domain" or regions homologous with a "PDZ domain".

As used herein, the term "PDZ domain" includes an amino acid sequence of about 80 to 90 amino acid residues in length and having a bit score for the alignment of the sequence to the PDZ domain (HMM) of at least 30. Preferably, a PDZ domain includes at least about 70 to 120 amino acids, more preferably about 80 to 110 amino acid residues, or about 80 to 90 amino acids and has a bit score for the alignment of the sequence to the PDZ domain (HMM) of at least 5, 10, 20, 25, 30, 32 or greater. The PDZ domain (HMM) has been assigned the PFAM Accession Number PF00595 (<http://genome.wustl.edu/Pfam/.html>). Preferably, a PDZ further includes a conserved glycine-phenylalanine dipeptide in the carboxylate ligand binding loop. An alignment of the PDZ domain (amino acids 890 to 975 of SEQ ID NO:2) of human 33521 with a consensus amino acid sequence (SEQ ID NO:6)

derived from a PFAM hidden Markov model is depicted in Figure 2C. An alignment of the PDZ domain (amino acids 900 to 976 of SEQ ID NO:2) of human 33521 with a consensus amino acid sequence (SEQ ID NO:11) derived from a SMART hidden Markov model is depicted in Figure 3C.

5 In a preferred embodiment 33521 polypeptide or protein has a "PDZ domain" or a region which includes at least about 70 to 120 more preferably about 80 to 110 or 80 to 90 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "PDZ domain," e.g., the PDZ domain of human 33521 (e.g., residues 890 to 975 of SEQ ID NO:2). The 33521 polypeptide as the conserved "GF" dipeptide motif at 10 about residues 903 to 904 of SEQ ID NO:2. To identify the presence of a "PDZ" domain in a 33521 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of Pfam HMMs , e.g., as described above. A "PDZ domain" was located by such a search in the amino acid sequence of human 33521 at about residues 890 to 975 of SEQ ID 15 NO:2 (see Example 1).

An RBD domain, or "Ras-binding" domain can be found on a variety of cell signalling molecules. The paradigm for this domain is the RBD of the Raf serine-threonine kinase, which is a key component of the Ras signalling pathway. Ras is a critical small GTPase which regulates cell proliferation. The central anti-parallel β -sheet of the RBD 20 interacts with two β -strands from Ras effector region by means of side chain and main chain interactions (Nassar *et al.* (1995) *Nature* 375:554-6).

33521 polypeptide can include a "RBD domain" or regions homologous with a "RBD domain".

As used herein, the term "RBD domain" includes an amino acid sequence of about 25 65 to 80 amino acid residues in length and having a bit score for the alignment of the sequence to the RBD domain consensus from the SMART database of HMM of at least 65. Preferably, a RBD domain includes at least about 40 to 100 amino acids, more preferably about 30 50 to 90 amino acid residues, or about 65 to 80 amino acids and has a bit score for the alignment of the sequence to the RBD domain consensus derived from the SMART database (HMM) of at least 10, 20, 30, 40, 50, 60 or greater. An alignment of the RBD domain (amino acids 810 to 873 of SEQ ID NO:2) of human 33521 with a consensus amino acid

sequence (SEQ ID NO:5) derived from a PFAM hidden Markov model is depicted in Figure 2B. An alignment of the RBD domain (amino acids 810 to 881 of SEQ ID NO:2) of human 33521 with a consensus amino acid sequence (SEQ ID NO:10) derived from a SMART hidden Markov model is depicted in Figure 3B.

5 In a preferred embodiment 33521 polypeptide or protein has a "RBD domain" or a region which includes at least about 40 to 100 more preferably about 50 to 90 or 65 to 80 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "RBD domain," e.g., the RBD domain of human 33521 (e.g., residues 810 to 881 of SEQ ID NO:2).

10 To identify the presence of a "RBD" domain in a 33521 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a SMART database (Simple Modular Architecture Research Tool, <http://smart.embl-heidelberg.de/>) of HMMs as described in Schultz *et al.* (1998), *Proc. Natl. Acad. Sci. USA* 95:5857 and Schultz *et al.* (2000) *Nucl. Acids Res* 28:231. The database contains domains identified by profiling with the hidden 15 Markov models of the HMMer2 search program (R. Durbin *et al.* (1998) *Biological sequence analysis: probabilistic models of proteins and nucleic acids*. Cambridge University Press.; <http://hmmer.wustl.edu/>). The database also is extensively annotated and monitored by experts to enhance accuracy. A "RBD domain" was located by such a search in the amino 20 acid sequence of human 33521 at about residues 810 to 880 of SEQ ID NO:2 (see Example 1).

A 33521 molecule can include: at least one and preferably two PH domains; an RBD domain; a PDZ domain; and a Rho GEF domain.

A 33521 molecule can further include at least one coiled-coil domain; at least one, 25 two, four, six, eight, ten, twenty, twenty-five, or preferably thirty protein kinase C phosphorylation sites; at least one, two, four, six, eight, ten, 20, 25, 30, 35, 40, or preferably 45 casein kinase II phosphorylation sites; at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or preferably twelve N-glycosylation sites; at least one, two, three, four, five, or preferably six cAMP-cGMP; at least one, two, three, four, five, six, seven, eight, 30 nine, ten, eleven, twelve, fifteen, eighteen, or preferably twenty predicted N-myristylation

sites; at least one predicted glycosaminoglycan site; and at least one, two, or preferably three tyrosine kinase phosphorylation sites.

As the 33521 polypeptides of the invention may modulate 33521-mediated activities, they may be useful as of for developing novel diagnostic and therapeutic agents for 33521-mediated or related disorders, as described below.

As used herein, a "33521 activity", "biological activity of 33521" or "functional activity of 33521", refers to an activity exerted by a 33521 protein, polypeptide or nucleic acid molecule. For example, a 33521 activity can be an activity exerted by 33521 in a physiological milieu on, e.g., a 33521-responsive cell or on a 33521 substrate, e.g., a protein substrate. A 33521 activity can be determined *in vivo* or *in vitro*. In one embodiment, a 33521 activity is a direct activity, such as an association with a 33521 target molecule. A "target molecule" or "binding partner" is a molecule with which a 33521 protein binds or interacts in nature, e.g., a Rac guanine nucleotide exchange factor (GEF).

A 33521 activity can also be an indirect activity, e.g., a cellular signaling activity mediated by interaction of the 33521 protein with a 33521 receptor. The features of the 33521 molecules of the present invention can provide similar biological activities as Rho GEF family members. For example, the 33521 proteins of the present invention can have one or more of the following activities: (1) modulation of cell morphology, e.g., regulation of focal adhesions, or stress fibers; (2) modulation of cell motility, e.g., regulation of lamellipodia, and leading edges; (3) modulation of cell adhesion, e.g., cadherin-mediated adhesion, or adherens junctions; (4) activation of small GTPases by stimulating guanine nucleotide exchange; (5) signaling in response to small lipophilic second messengers, e.g., phosphoinositols, or ascorbyl stereates; (6) signaling in response to phosphorylation events, e.g., phosphorylation of 3352 predicted tyrosine phosphorylation sites or predicted protein kinase C phosphorylation sites; (7) signaling in response to cell surface receptors, e.g., CD44; and (8) signaling in response to activation of Ras or G $\beta\gamma$ proteins.

Thus, the 33521 molecules can act as novel diagnostic targets and therapeutic agents for controlling cell proliferation and differentiation disorders, including metastatic disorders.

Examples of cellular proliferative and/or differentiative disorders include cancer, e.g., carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, e.g.,

leukemias. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast and liver origin.

As used herein, the terms "cancer", "hyperproliferative" and "neoplastic" refer to cells having the capacity for autonomous growth. Examples of such cells include cells having an 5 abnormal state or condition characterized by rapidly proliferating cell growth.

Hyperproliferative and neoplastic disease states may be categorized as pathologic, i.e., characterizing or constituting a disease state, or may be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly 10 transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair.

The terms "cancer" or "neoplasms" include malignancies of the various organ 15 systems, such as affecting lung, breast, thyroid, lymphoid, gastrointestinal, and genitourinary tract, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

The term "carcinoma" is art recognized and refers to malignancies of epithelial or 20 endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, e.g., which include malignant tumors 25 composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

The term "sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation.

30 Additional examples of proliferative disorders include hematopoietic neoplastic disorders. As used herein, the term "hematopoietic neoplastic disorders" includes diseases

involving hyperplastic/neoplastic cells of hematopoietic origin. A hematopoietic neoplastic disorder can arise from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Preferably, the diseases arise from poorly differentiated acute leukemias, e.g., erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders
5 include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) *Crit Rev. in Oncol./Hematol.* 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia
10 (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Sternberg disease.

15 The 33521 protein, fragments thereof, and derivatives and other variants of the sequence in SEQ ID NO:2 thereof are collectively referred to as "polypeptides or proteins of the invention" or "33521 polypeptides or proteins". Nucleic acid molecules encoding such polypeptides or proteins are collectively referred to as "nucleic acids of the invention" or "33521 nucleic acids." 33521 molecules refer to 33521 nucleic acids, polypeptides, and
20 antibodies.

As used herein, the term "nucleic acid molecule" includes DNA molecules (e.g., a cDNA or genomic DNA), RNA molecules (e.g., an mRNA) and analogs of the DNA or RNA. A DNA or RNA analog can be synthesized from nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

25 The term "isolated nucleic acid molecule" or "purified nucleic acid molecule" includes nucleic acid molecules that are separated from other nucleic acid molecules present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid
30 is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the

nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid 5 molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and 10 washing. Guidance for performing hybridization reactions can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, which is incorporated by reference. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at 15 about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; 20 and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified.

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under a 25 stringency condition described herein to the sequence of SEQ ID NO:1 or SEQ ID NO:3, corresponds to a naturally-occurring nucleic acid molecule.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature. For example a naturally occurring nucleic acid molecule can encode a natural protein.
30 As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include at least an open reading frame encoding a 33521 protein. The gene can

optionally further include non-coding sequences, e.g., regulatory sequences and introns. Preferably, a gene encodes a mammalian 33521 protein or derivative thereof.

An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. "Substantially free" means that a preparation of 33521 protein is at least 10% pure. In a preferred embodiment, the preparation of 33521 protein has less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-33521 protein (also referred to herein as a "contaminating protein"), or of chemical precursors or non-33521 chemicals. When the 33521 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 33521 without abolishing or substantially altering a 33521 activity. Preferably the alteration does not substantially alter the 33521 activity, e.g., the activity is at least 20%, 40%, 60%, 70% or 80% of wild-type. An "essential" amino acid residue is a residue that, when altered from the wild-type sequence of 33521, results in abolishing a 33521 activity such that less than 20% of the wild-type activity is present. For example, conserved amino acid residues in 33521 are predicted to be particularly unamenable to alteration.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a

33521 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 33521 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 33521 biological activity to identify mutants that retain 5 activity. Following mutagenesis of SEQ ID NO:1 or SEQ ID NO:3, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

As used herein, a "biologically active portion" of a 33521 protein includes a fragment of a 33521 protein which participates in an interaction, e.g., an intramolecular or an inter-molecular interaction. An inter-molecular interaction can be a specific binding interaction or 10 an enzymatic interaction (e.g., the interaction can be transient and a covalent bond is formed or broken). An inter-molecular interaction can be between a 33521 molecule and a non-33521 molecule or between a first 33521 molecule and a second 33521 molecule (e.g., a dimerization interaction). Biologically active portions of a 33521 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid 15 sequence of the 33521 protein, e.g., the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length 33521 proteins, and exhibit at least one activity of a 33521 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 33521 protein, e.g., guanine nucleotide exchange. A biologically active portion of a 33521 protein can be a polypeptide which is, for example, 10, 25, 50, 100, 20 200 or more amino acids in length. Biologically active portions of a 33521 protein can be used as targets for developing agents which modulate a 33521 mediated activity, e.g., guanine nucleotide exchange.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

25 To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for 30 comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference

sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology").

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) *J. Mol. Biol.* 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller ((1989) CABIOS, 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to 33521 nucleic acid molecules of the

invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to 33521 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25:3389-3402.

5 When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

Particularly preferred 33521 polypeptides of the present invention have an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO:2. In the context 10 of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences 15 that contain a common structural domain having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:2 are termed substantially identical.

In the context of nucleotide sequence, the term "substantially identical" is used herein 20 to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common 25 functional polypeptide activity. For example, nucleotide sequences having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:1 or 3 are termed substantially identical.

"Misexpression or aberrant expression", as used herein, refers to a non-wildtype 30 pattern of gene expression at the RNA or protein level. It includes: expression at non-wild type levels, i.e., over- or under-expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a

pattern of expression that differs from wild type in terms of altered, e.g., increased or decreased, expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, translated amino acid sequence, post-translational modification, or biological activity of the
5 expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

"Subject," as used herein, refers to human and non-human animals. The term "non-
10 human animals" of the invention includes all vertebrates, e.g., mammals, such as non-human primates (particularly higher primates), sheep, dog, rodent (e.g., mouse or rat), guinea pig, goat, pig, cat, rabbits, cow, and non-mammals, such as chickens, amphibians, reptiles, etc. In a preferred embodiment, the subject is a human. In another embodiment, the subject is an experimental animal or animal suitable as a disease model.

15 A "purified preparation of cells", as used herein, refers to an in vitro preparation of cells. In the case cells from multicellular organisms (e.g., plants and animals), a purified preparation of cells is a subset of cells obtained from the organism, not the entire intact organism. In the case of unicellular microorganisms (e.g., cultured cells and microbial cells), it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

20 Various aspects of the invention are described in further detail below.

Isolated Nucleic Acid Molecules

In one aspect, the invention provides, an isolated or purified, nucleic acid molecule
that encodes a 33521 polypeptide described herein, e.g., a full-length 33521 protein or a
fragment thereof, e.g., a biologically active portion of 33521 protein. Also included is a
25 nucleic acid fragment suitable for use as a hybridization probe, which can be used, e.g., to
identify a nucleic acid molecule encoding a polypeptide of the invention, 33521 mRNA, and
fragments suitable for use as primers, e.g., PCR primers for the amplification or mutation of
nucleic acid molecules.

30 In one embodiment, an isolated nucleic acid molecule of the invention includes the
nucleotide sequence shown in SEQ ID NO:1, or a portion of any of these nucleotide
sequences. In one embodiment, the nucleic acid molecule includes sequences encoding the

human 33521 protein (i.e., "the coding region" of SEQ ID NO:1, as shown in SEQ ID NO:3), as well as 5' untranslated sequences. Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 (e.g., SEQ ID NO:3) and, e.g., no flanking sequences which normally accompany the subject sequence. In another embodiment, the 5 nucleic acid molecule encodes a sequence corresponding to a fragment of the protein from about amino acid 1103 to 1292 of SEQ ID NO:2. In still other embodiments, the nucleic acid molecule encodes a sequence corresponding to one of the following fragments of the 33521 protein: from about amino acid 1 to 507, 507 to 620, 620 to 810, 810 to 853, 853 to 890, 890 to 975, 975 to 1103, 1292 to 1353, and 1353 to 1455, of SEQ ID NO:2.

10 In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or a portion of any of these nucleotide sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, such that it can hybridize 15 (e.g., under a stringency condition described herein) to the nucleotide sequence shown in SEQ ID NO:1 or 3, thereby forming a stable duplex.

20 In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence which is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the entire length of the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or a portion, preferably of the same length, of any of these nucleotide sequences.

33521 Nucleic Acid Fragments

A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO:1 or 3. For example, such a nucleic acid molecule can include 25 a fragment which can be used as a probe or primer or a fragment encoding a portion of a 33521 protein, e.g., an immunogenic or biologically active portion of a 33521 protein. A fragment can comprise those nucleotides of SEQ ID NO:1, which encode a Rho GEF domain of human 33521. Other fragments include those encoding a PH domain, a PDZ domain, or a RBD domain of human 33521. The nucleotide sequence determined from the cloning of the 30 33521 gene allows for the generation of probes and primers designed for use in identifying

and/or cloning other 33521 family members, or fragments thereof, as well as 33521 homologues, or fragments thereof, from other species.

In another embodiment, a nucleic acid includes a nucleotide sequence that includes part, or all, of the coding region and extends into either (or both) the 5' or 3' noncoding region. Other 5 embodiments include a fragment which includes a nucleotide sequence encoding an amino acid fragment described herein. Nucleic acid fragments can encode a specific domain or site described herein or fragments thereof, particularly fragments thereof which are at least 50 amino acids in length, more preferably 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1650, 1670, 1680, 1685, 1690, 1695, 1700, or 1701 amino acids in 10 length.

Fragments also include nucleic acid sequences corresponding to specific amino acid sequences described above or fragments thereof. Nucleic acid fragments should not to be construed as encompassing those fragments that may have been disclosed prior to the invention.

A nucleic acid fragment can include a sequence corresponding to a domain, region, or 15 functional site described herein. A nucleic acid fragment can also include one or more domain, region, or functional site described herein. Thus, for example, a 33521 nucleic acid fragment can include a sequence corresponding to a Rho GEF domain, a PH domain, a PDZ domain, or an RBD domain.

33521 probes and primers are provided. Typically a probe/primer is an isolated or 20 purified oligonucleotide. The oligonucleotide typically includes a region of nucleotide sequence that hybridizes under a stringency condition described herein to at least about 7, 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense or antisense sequence of SEQ ID NO:1 or SEQ ID NO:3, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or SEQ ID NO:3. 25 Preferably, an oligonucleotide is less than about 200, 150, 120, or 100 nucleotides in length.

In one embodiment, the probe or primer is attached to a solid support, e.g., a solid support described herein.

One exemplary kit of primers includes a forward primer that anneals to the coding strand and a reverse primer that anneals to the non-coding strand. The forward primer can 30 anneal to the start codon, e.g., the nucleic acid sequence encoding amino acid residue 1 of SEQ ID NO:2. The reverse primer can anneal to the ultimate codon, e.g., the codon

immediately before the stop codon, e.g., the codon encoding amino acid residue 1701 of SEQ ID NO:2. In a preferred embodiment, the annealing temperatures of the forward and reverse primers differ by no more than 5, 4, 3, or 2°C.

In a preferred embodiment the nucleic acid is a probe which is at least 10, 12, 15, 18, 5 20 and less than 200, more preferably less than 100, or less than 50, nucleotides in length. It should be identical, or differ by 1, or 2, or less than 5 or 10 nucleotides, from a sequence disclosed herein. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

10 A probe or primer can be derived from the sense or anti-sense strand of a nucleic acid which encodes: a Rho GEF domain, a PH domain, a PDZ domain, or a RBD domain of human 33521.

In another embodiment a set of primers is provided, e.g., primers suitable for use in a PCR, which can be used to amplify a selected region of a 33521 sequence, e.g., a domain, 15 region, site or other sequence described herein. The primers should be at least 5, 10, or 50 base pairs in length and less than 100, or less than 200, base pairs in length. The primers should be identical, or differs by one base from a sequence disclosed herein or from a naturally occurring variant. For example, primers suitable for amplifying all or a portion of any of the following regions are provided: a Rho GEF domain from about amino acid 1103 20 to 1292 of SEQ ID NO:2; a PH domain from about amino acid 507 to 622 and 1353 to 1457 of SEQ ID NO:2; an RBD domain from about amino acid 810 to 881 of SEQ ID NO:2; or a PDZ domain from about amino acid 890 to 975 of SEQ ID NO:2.

A nucleic acid fragment can encode an epitope bearing region of a polypeptide described herein.

25 A nucleic acid fragment encoding a "biologically active portion of a 33521 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1 or 3, which encodes a polypeptide having a 33521 biological activity (e.g., the biological activities of the 33521 proteins are described herein), expressing the encoded portion of the 33521 protein (e.g., by recombinant expression *in vitro*) and assessing the 30 activity of the encoded portion of the 33521 protein. For example, a nucleic acid fragment encoding a biologically active portion of 33521 includes a Rho GEF domain, e.g., amino acid

residues about 1103 to 1292 of SEQ ID NO:2. A nucleic acid fragment encoding a biologically active portion of a 33521 polypeptide, may comprise a nucleotide sequence which is greater than 300 or more nucleotides in length.

In preferred embodiments, a nucleic acid includes a nucleotide sequence which is
5 about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1500, 2000, 2500, 3000,
3500, 4000, 4500, 5000 or more nucleotides in length and hybridizes under a stringency
condition described herein to a nucleic acid molecule of SEQ ID NO:1 or SEQ ID NO:3.

In preferred embodiments, the fragment includes at least one, and preferably at least
5, 10, 15, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1500,
10 2000, 2500, 3000, 3500, 4000, or 4500 nucleotides from nucleotides 1-2142, 1-2273, 1-3415,
1-4822, 1-4937, 1-4949, 1-4958, or 1-4973 of SEQ ID NO:1.

In preferred embodiments, the fragment includes the nucleotide sequence of SEQ ID
NO:3 and at least one, and preferably at least 5, 10, 15, 25, 50, 75, 100, 150, 200, or 240
consecutive nucleotides of SEQ ID NO:1.

15 In preferred embodiments, the fragment includes at least one, and preferably at least
5, 10, 15, 25, 50, 75, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300,
1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, or more nucleotides encoding a protein
including at least 5, 10, 15, 20, 25, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 500, 600,
700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, or 1700 consecutive amino acids
20 of SEQ ID NO:2. In one embodiment, the encoded protein includes at least 5, 10, 15, 20, 25,
30, 40, 50, 100, 150, 200, 250, 300, 400, 500, or 600 consecutive amino acids from residues
1-631 of SEQ ID NO:2.

In preferred embodiments, the nucleic acid fragment includes a nucleotide sequence
that is other than, e.g., differs by at least one, two, three or more nucleotides from, a sequence
25 in WO 00/40607 or in GenBank™ Accession numbers AI094945, AI126294, AW338968,
AW026228, AI982584, AI589050, BE504999, AL122086, AF120323, AF120324,
AF195656.

In preferred embodiments, the fragment comprises the coding region of 33521, e.g.,
the nucleotide sequence of SEQ ID NO:6.

33521 Nucleic Acid Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid which encodes the same 33521 proteins as those encoded by the nucleotide sequence disclosed herein. In another embodiment; an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that shown in SEQ ID NO:2. If alignment is needed for this comparison the sequences should be aligned for maximum homology. The encoded protein can differ by no more than 5, 4, 3, 2, or 1 amino acid. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Nucleic acids of the inventor can be chosen for having codons, which are preferred, or non-preferred, for a particular expression system. E.g., the nucleic acid can be one in which at least one codon, at preferably at least 10%, or 20% of the codons has been altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, or CHO cells.

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO: 1 or 3, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the nucleotides in the subject nucleic acid. The nucleic acid can differ by no more than 5, 4, 3, 2, or 1 nucleotide. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least

about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more identical to the nucleotide sequence shown in SEQ ID NO:2 or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under a stringency condition described herein, to the nucleotide sequence shown in SEQ ID NO 2 or a fragment of the sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the 33521 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 33521 gene.

Preferred variants include those that are correlated with guanine nucleotide exchange activity.

Allelic variants of 33521, e.g., human 33521, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the 33521 protein within a population that maintain the ability to bind a Rho family protein, e.g., Rac. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the 33521, e.g., human 33521, protein within a population that do not have the ability to bind a Rho family protein, e.g., Rac.. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2, or a substitution, insertion, or deletion in critical residues or critical regions of the protein.

Moreover, nucleic acid molecules encoding other 33521 family members and, thus, which have a nucleotide sequence which differs from the 33521 sequences of SEQ ID NO:1 or SEQ ID NO:3 are intended to be within the scope of the invention.

Antisense Nucleic Acid Molecules, Ribozymes and Modified 33521 Nucleic Acid Molecules

In another aspect, the invention features, an isolated nucleic acid molecule which is antisense to 33521. An "antisense" nucleic acid can include a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire 33521 coding

strand, or to only a portion thereof (e.g., the coding region of human 33521 corresponding to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 33521 (e.g., the 5' and 3' untranslated regions).

5 An antisense nucleic acid can be designed such that it is complementary to the entire coding region of 33521 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 33521 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 33521 mRNA, e.g., between the -10 and +10 regions of the target gene

10 nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject (e.g., by direct injection at a tissue site), or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 33521 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation.

Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be

delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. A ribozyme having specificity for a 33521-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of a 33521 cDNA disclosed herein (i.e., SEQ ID NO:1 or SEQ ID NO:3), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach (1988) *Nature* 334:585-591). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 33521-encoding mRNA. See, e.g., Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, 33521 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

33521 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 33521 (e.g., the 33521 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 33521 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6:569-84; Helene, C. i (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14:807-15. The potential sequences that can be targeted for triple helix formation can be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand

of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or
5 colorimetric.

A 33521 nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For non-limiting examples of synthetic oligonucleotides with modifications see Toulmé (2001) *Nature Biotech.* 19:17 and Faria *et al.* (2001) *Nature Biotech.* 19:40-44. Such
10 phosphoramidite oligonucleotides can be effective antisense agents.

For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4: 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, e.g., a DNA mimic, in which the deoxyribose phosphate
15 backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra* and Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93: 14670-675.

20 PNAs of 33521 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 33521 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping);
25 as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. *et al.* (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport
30 across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT

Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (see, e.g., Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (see, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may 5 be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

The invention also includes molecular beacon oligonucleotide primer and probe molecules having at least one region which is complementary to a 33521 nucleic acid of the invention, two complementary regions one having a fluorophore and one a quencher such 10 that the molecular beacon is useful for quantitating the presence of the 33521 nucleic acid of the invention in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi *et al.*, U.S. Patent No. 5,854,033; Nazarenko *et al.*, U.S. Patent No. 5,866,336, and Livak *et al.*, U.S. Patent 5,876,930.

Isolated 33521 Polypeptides

15 In another aspect, the invention features, an isolated 33521 protein, or fragment, e.g., a biologically active portion, for use as immunogens or antigens to raise or test (or more generally to bind) anti-33521 antibodies. 33521 protein can be isolated from cells or tissue sources using standard protein purification techniques. 33521 protein or fragments thereof can be produced by recombinant DNA techniques or synthesized chemically.

20 Polypeptides of the invention include those which arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and post-translational events. The polypeptide can be expressed in systems, e.g., cultured cells, which result in substantially the same post-translational modifications present when expressed the polypeptide is expressed in a native cell, or in 25 systems which result in the alteration or omission of post-translational modifications, e.g., glycosylation or cleavage, present when expressed in a native cell.

In a preferred embodiment, a 33521 polypeptide has one or more of the following characteristics:

- (i) has the ability to stimulate guanine nucleotide exchange of a Rho protein, e.g.,
30 Rac;

- (ii) it has a molecular weight, e.g., a deduced molecular weight, preferably ignoring any contribution of post translational modifications, amino acid composition or other physical characteristic of SEQ ID NO:2;
- (iii) it has an overall sequence similarity of at least 50%, preferably at least 60%,
5 more preferably at least 70, 80, 90, or 95%, with a polypeptide a of SEQ ID NO:2;
- (iv) it has a Rho GEF domain which is preferably about 70%, 80%, 90% or 95% with amino acid residues about 1103 to 1292 of SEQ ID NO:2;
- (v) it has two PH domains which are preferably about 70%, 80%, 90% or 95% with amino acid residues about 507 to 620, and 1353 to 1455, of SEQ ID NO:2;
10 (vi) it has a RBD domain which is preferably about 70%, 80%, 90% or 95% with amino acid residues about 810 to 881 of SEQ ID NO:2; or
- (vii) it has a PDZ domain which is preferably about 70%, 80%, 90% or 95% with amino acid residues about 890 to 975 of SEQ ID NO:2.

In a preferred embodiment the 33521 protein, or fragment thereof, differs from the corresponding sequence in SEQ ID:2. In one embodiment it differs by at least one but by less than 15, 10 or 5 amino acid residues. In another it differs from the corresponding sequence in SEQ ID NO:2 by at least one residue but less than 20%, 15%, 10% or 5% of the residues in it differ from the corresponding sequence in SEQ ID NO:2. (If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) The differences are, preferably, differences or changes at a non essential residue or a conservative substitution. In a preferred embodiment the differences are not in the Rho GEF domain. In another preferred embodiment one or more differences are in the Rho GEF domain.
20

Other embodiments include a protein that contain one or more changes in amino acid sequence, e.g., a change in an amino acid residue which is not essential for activity. Such 33521 proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity.

In one embodiment, the protein includes an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to SEQ ID
30 NO:2.

A 33521 protein or fragment is provided which varies from the sequence of SEQ ID NO:2 in regions defined by amino acids about 1 to 1102, and 1293 to 1701 by at least one but by less than 15, 10 or 5 amino acid residues in the protein or fragment but which does not differ from SEQ ID NO:2 in regions defined by amino acids about 1103 to 1292. (If this comparison requires alignment the sequences should be aligned for maximum homology.

5 "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) In some embodiments the difference is at a non-essential residue or is a conservative substitution, while in others the difference is at an essential residue or is a non-conservative substitution.

10 In one embodiment, a biologically active portion of a 33521 protein includes a Rho GEF domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 33521 protein.

15 In a preferred embodiment, the 33521 protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the 33521 protein is substantially identical to SEQ ID NO:2. In yet another embodiment, the 33521 protein is substantially identical to SEQ ID NO:2 and retains the functional activity of the protein of SEQ ID NO:2, as described in detail in the subsections above.

33521 Chimeric or Fusion Proteins

20 In another aspect, the invention provides 33521 chimeric or fusion proteins. As used herein, a 33521 "chimeric protein" or "fusion protein" includes a 33521 polypeptide linked to a non-33521 polypeptide. A "non-33521 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 33521 protein, e.g., a protein which is different from the 33521 protein and which is derived 25 from the same or a different organism. The 33521 polypeptide of the fusion protein can correspond to all or a portion e.g., a fragment described herein of a 33521 amino acid sequence. In a preferred embodiment, a 33521 fusion protein includes at least one (or two) biologically active portion of a 33521 protein. The non-33521 polypeptide can be fused to the N-terminus or C-terminus of the 33521 polypeptide.

30 The fusion protein can include a moiety which has a high affinity for a ligand. For example, the fusion protein can be a GST-33521 fusion protein in which the 33521

sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 33521. Alternatively, the fusion protein can be a 33521 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of 33521 can be increased 5 through use of a heterologous signal sequence.

Fusion proteins can include all or a part of a serum protein, e.g., an IgG constant region, or human serum albumin.

The 33521 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 33521 fusion proteins can be used to 10 affect the bioavailability of a 33521 substrate. 33521 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 33521 protein; (ii) mis-regulation of the 33521 gene; and (iii) aberrant post-translational modification of a 33521 protein.

Moreover, the 33521-fusion proteins of the invention can be used as immunogens to 15 produce anti-33521 antibodies in a subject, to purify 33521 ligands and in screening assays to identify molecules which inhibit the interaction of 33521 with a 33521 substrate.

Expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A 33521-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 33521 protein.

20 Variants of 33521 Proteins

In another aspect, the invention also features a variant of a 33521 polypeptide, e.g., which functions as an agonist (mimetics) or as an antagonist. Variants of the 33521 proteins can be generated by mutagenesis, e.g., discrete point mutation, the insertion or deletion of sequences or the truncation of a 33521 protein. An agonist of the 33521 proteins can retain 25 substantially the same, or a subset, of the biological activities of the naturally occurring form of a 33521 protein. An antagonist of a 33521 protein can inhibit one or more of the activities of the naturally occurring form of the 33521 protein by, for example, competitively modulating a 33521-mediated activity of a 33521 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Preferably, treatment of a 30 subject with a variant having a subset of the biological activities of the naturally occurring

form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 33521 protein.

Variants of a 33521 protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a 33521 protein for agonist or antagonist activity.

5 Libraries of fragments e.g., N terminal, C terminal, or internal fragments, of a 33521 protein coding sequence can be used to generate a variegated population of fragments for screening and subsequent selection of variants of a 33521 protein. Variants in which a cysteine residues is added or deleted or in which a residue which is glycosylated is added or deleted are particularly preferred.

10 Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property are known in the art. Such methods are adaptable for rapid screening of the gene libraries generated by combinatorial mutagenesis of 33521 proteins. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional 15 mutants in the libraries, can be used in combination with the screening assays to identify 33521 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6:327-331).

Cell based assays can be exploited to analyze a variegated 33521 library. For example, a library of expression vectors can be transfected into a cell line, e.g., a cell line, 20 which ordinarily responds to 33521 in a substrate-dependent manner. The transfected cells are then contacted with 33521 and the effect of the expression of the mutant on signaling by the 33521 substrate can be detected, e.g., by measuring stimulation of guanine nucleotide exchange on a Rho protein. Plasmid DNA can then be recovered from the cells which score 25 for inhibition, or alternatively, potentiation of signaling by the 33521 substrate, and the individual clones further characterized.

In another aspect, the invention features a method of making a 33521 polypeptide, e.g., a peptide having a non-wild type activity, e.g., an antagonist, agonist, or super agonist of a naturally occurring 33521 polypeptide, e.g., a naturally occurring 33521 polypeptide. The method includes: altering the sequence of a 33521 polypeptide, e.g., altering the sequence, 30 e.g., by substitution or deletion of one or more residues of a non-conserved region, a domain or residue disclosed herein, and testing the altered polypeptide for the desired activity.

In another aspect, the invention features a method of making a fragment or analog of a 33521 polypeptide a biological activity of a naturally occurring 33521 polypeptide. The method includes: altering the sequence, e.g., by substitution or deletion of one or more residues, of a 33521 polypeptide, e.g., altering the sequence of a non-conserved region, or a 5 domain or residue described herein, and testing the altered polypeptide for the desired activity.

Anti-33521 Antibodies

In another aspect, the invention provides an anti-33521 antibody, or a fragment thereof (e.g., an antigen-binding fragment thereof). The term "antibody" as used herein 10 refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. As used herein, the term "antibody" refers to a protein comprising at least one, and preferably two, heavy (H) chain variable regions (abbreviated herein as VH), and at least one and preferably two light (L) chain variable regions (abbreviated herein as VL). The VH and VL regions can be further subdivided into regions of hypervariability, 15 termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" (FR). The extent of the framework region and CDR's has been precisely defined (see, Kabat, E.A., et al. (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, which are 20 incorporated herein by reference). Each VH and VL is composed of three CDR's and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The anti-33521 antibody can further include a heavy and light chain constant region, to thereby form a heavy and light immunoglobulin chain, respectively. In one embodiment, 25 the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are interconnected by, e.g., disulfide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that 30 interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune

system (e.g., effector cells) and the first component (C1q) of the classical complement system.

As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 KDa or 214 amino acids) are encoded by a variable region gene at the NH₂-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH-terminus. Full-length immunoglobulin "heavy chains" (about 50 KDa or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

The term "antigen-binding fragment" of an antibody (or simply "antibody portion," or "fragment"), as used herein, refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to the antigen, e.g., 33521 polypeptide or fragment thereof. Examples of antigen-binding fragments of the anti-33521 antibody include, but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with

skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The anti-33521 antibody can be a polyclonal or a monoclonal antibody. In other embodiments, the antibody can be recombinantly produced, e.g., produced by phage display or by combinatorial methods.

Phage display and combinatorial methods for generating anti-33521 antibodies are known in the art (as described in, e.g., Ladner et al. U.S. Patent No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791; Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No. WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum Antibod Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J Mol Biol* 226:889-896; Clackson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *PNAS* 89:3576-3580; Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc Acid Res* 19:4133-4137; and Barbas et al. (1991) *PNAS* 88:7978-7982, the contents of all of which are incorporated by reference herein).

In one embodiment, the anti-33521 antibody is a fully human antibody (e.g., an antibody made in a mouse which has been genetically engineered to produce an antibody from a human immunoglobulin sequence), or a non-human antibody, e.g., a rodent (mouse or rat), goat, primate (e.g., monkey), camel antibody. Preferably, the non-human antibody is a rodent (mouse or rat antibody). Method of producing rodent antibodies are known in the art.

Human monoclonal antibodies can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, e.g., Wood et al. International Application WO 91/00906, Kucherlapati et al. PCT publication WO 91/10741; Lonberg et al. International Application WO 92/03918; Kay et al. International Application 92/03917; Lonberg, N. et al. 1994 *Nature* 368:856-859; Green,

L.L. et al. 1994 *Nature Genet.* 7:13-21; Morrison, S.L. et al. 1994 *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Bruggeman et al. 1993 *Year Immunol* 7:33-40; Tuailon et al. 1993 *PNAS* 90:3720-3724; Bruggeman et al. 1991 *Eur J Immunol* 21:1323-1326).

An anti-33521 antibody can be one in which the variable region, or a portion thereof, 5 e.g., the CDR's, are generated in a non-human organism, e.g., a rat or mouse. Chimeric, CDR-grafted, and humanized antibodies are within the invention. Antibodies generated in a non-human organism, e.g., a rat or mouse, and then modified, e.g., in the variable framework or constant region, to decrease antigenicity in a human are within the invention.

Chimeric antibodies can be produced by recombinant DNA techniques known in the 10 art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted (see Robinson et al., International Patent Publication PCT/US86/02269; Akira, et al., European Patent Application 184,187; Taniguchi, M., European Patent 15 Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Application WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988 *Science* 240:1041-1043); Liu et al. (1987) *PNAS* 84:3439-3443; Liu et al., 1987, *J. Immunol.* 139:3521-3526; Sun et al. (1987) *PNAS* 84:214-218; Nishimura et al., 1987, *Canc. Res.* 47:999-1005; Wood et al. 20 (1985) *Nature* 314:446-449; and Shaw et al., 1988, *J. Natl Cancer Inst.* 80:1553-1559).

A humanized or CDR-grafted antibody will have at least one or two but generally all three recipient CDR's (of heavy and or light immunoglobulin chains) replaced with a donor CDR. The antibody may be replaced with at least a portion of a non-human CDR or only some of the CDR's may be replaced with non-human CDR's. It is only necessary to replace 25 the number of CDR's required for binding of the humanized antibody to a 33521 or a fragment thereof. Preferably, the donor will be a rodent antibody, e.g., a rat or mouse antibody, and the recipient will be a human framework or a human consensus framework. Typically, the immunoglobulin providing the CDR's is called the "donor" and the immunoglobulin providing the framework is called the "acceptor." In one embodiment, the 30 donor immunoglobulin is a non-human (e.g., rodent). The acceptor framework is a naturally-

occurring (e.g., a human) framework or a consensus framework, or a sequence about 85% or higher, preferably 90%, 95%, 99% or higher identical thereto.

As used herein, the term "consensus sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See e.g.,

5 Winnacker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A "consensus framework" refers to the framework region in the consensus immunoglobulin sequence.

10 An antibody can be humanized by methods known in the art. Humanized antibodies can be generated by replacing sequences of the Fv variable region which are not directly involved in antigen binding with equivalent sequences from human Fv variable regions.

General methods for generating humanized antibodies are provided by Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986, *BioTechniques* 4:214, and by Queen et al. US
15 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby incorporated by reference. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from a hybridoma producing an
20 antibody against a 33521 polypeptide or fragment thereof. The recombinant DNA encoding the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

Humanized or CDR-grafted antibodies can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDR's of an immunoglobulin chain can be replaced.

25 See e.g., U.S. Patent 5,225,539; Jones et al. 1986 *Nature* 321:552-525; Verhoeyan et al. 1988 *Science* 239:1534; Beidler et al. 1988 *J. Immunol.* 141:4053-4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference. Winter describes a CDR-grafting method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987; Winter US
30 5,225,539), the contents of which is expressly incorporated by reference.

Also within the scope of the invention are humanized antibodies in which specific amino acids have been substituted, deleted or added. Preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, a humanized antibody will have framework residues identical to the donor framework residue or to another amino acid other than the recipient framework residue. To generate such antibodies, a selected, small number of acceptor framework residues of the humanized immunoglobulin chain can be replaced by the corresponding donor amino acids. Preferred locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (see e.g., US 5,585,089). Criteria for selecting amino acids from the donor are described in US 5,585,089, e.g., columns 12-16 of US 5,585,089, the e.g., columns 12-16 of US 5,585,089, the contents of which are hereby incorporated by reference. Other techniques for humanizing antibodies are described in Padlan et al. EP 519596 A1, published on December 23, 1992.

In preferred embodiments an antibody can be made by immunizing with purified 33521 antigen, or a fragment thereof, e.g., a fragment described herein.

A full-length 33521 protein or, antigenic peptide fragment of 33521 can be used as an immunogen or can be used to identify anti-33521 antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. The antigenic peptide of 33521 should include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of 33521. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Fragments of 33521 which include residues about amino acid 741 to 750, from about 756 to 762, and from about 1363 to 1372, of SEQ ID NO:2; can be used to make, e.g., used as immunogens or used to characterize the specificity of an antibody, antibodies against hydrophilic regions of the 33521 protein. Similarly, fragments of 33521 which include residues about amino acid 722 to 730, from about 883 to 891, and from about 966 to 975, of SEQ ID NO:2 can be used to make an antibody against a hydrophobic region of the 33521 protein; a fragment of 33521 which includes residues 507 to 620 and 1353 to 1455 of SEQ ID NO:2 can be used to make an antibody against the PH domains of the 33521 protein; a fragment of 33521 which includes residues about 810 to 881 of SEQ ID NO:2 can be used to

make an antibody against an RBD domain of the 33521 protein; a fragment of 33521 which includes residues about 1103 to 1292 of SEQ ID NO:2 can be used to make an antibody against the Rho GEF region of the 33521 protein; a fragment of 33521 which includes residues about 890 to 975 of SEQ ID NO:2 can be used to make an antibody against the PDZ 5 region of the 33521 protein.

Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided.

Antibodies which bind only native 33521 protein, only denatured or otherwise non-native 33521 protein, or which bind both, are within the invention. Antibodies with linear or 10 conformational epitopes are within the invention. Conformational epitopes can sometimes be identified by identifying antibodies which bind to native but not denatured 33521 protein.

Preferred epitopes encompassed by the antigenic peptide are regions of 33521 are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human 33521 protein 15 sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the 33521 protein and are thus likely to constitute surface residues useful for targeting antibody production.

In a preferred embodiment the antibody can bind to the Rho GEF portion of the 33521 protein. In another embodiment, the antibody binds a PH domain, a PDZ domain, or 20 RBD domain portion of the 33521 protein.

The anti-33521 antibody can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Colcher, D. *et al.* (1999) *Ann NY Acad Sci* 880:263-80; and Reiter, Y. (1996) *Clin Cancer Res* 2:245-52). The single chain antibody can 25 be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target 33521 protein.

In a preferred embodiment the antibody has effector function and/or can fix complement. In other embodiments the antibody does not recruit effector cells; or fix complement.

In a preferred embodiment, the antibody has reduced or no ability to bind an Fc 30 receptor. For example, it is a isotype or subtype, fragment or other mutant, which does not

support binding to an Fc receptor, e.g., it has a mutagenized or deleted Fc receptor binding region.

In a preferred embodiment, an anti-33521 antibody alters (e.g., increases or decreases) the guanine nucleotide exchange activity of a 33521 polypeptide.

5 The antibody can be coupled to a toxin, e.g., a polypeptide toxin, e.g., ricin or diphtheria toxin or active fragment hereof, or a radioactive nucleus, or imaging agent, e.g., a radioactive, enzymatic, or other, e.g., imaging agent, e.g., a NMR contrast agent. Labels which produce detectable radioactive emissions or fluorescence are preferred.

An anti-33521 antibody (e.g., monoclonal antibody) can be used to isolate 33521 by 10 standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-33521 antibody can be used to detect 33521 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein.

Anti-33521 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to determine the efficacy of a given treatment regimen.

15 Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labelling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of 20 suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable 25 radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

The invention also includes a nucleic acid which encodes an anti-33521 antibody, e.g., an anti-33521 antibody described herein. Also included are vectors which include the nucleic acid and cells transformed with the nucleic acid, particularly cells which are useful for producing an antibody, e.g., mammalian cells, e.g. CHO or lymphatic cells.

The invention also includes cell lines, e.g., hybridomas, which make an anti-33521 antibody, e.g., and antibody described herein, and method of using said cells to make a 33521 antibody.

Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

5 In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide described herein. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors 10 include, e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses.

A vector can include a 33521 nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control 15 elements (e.g., polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby 20 produce proteins or polypeptides, including fusion proteins or polypeptides, encoded by nucleic acids as described herein (e.g., 33521 proteins, mutant forms of 33521 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of 33521 proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the 25 invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, (1990) *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

30 Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or

non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be used in 33521 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for 33521 proteins. In a preferred embodiment, a fusion protein expressed in a retroviral expression vector of the present invention can be used to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six weeks).

To maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., (1990) *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

The 33521 expression vector can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector or a vector suitable for expression in mammalian cells.

When used in mammalian cells, the expression vector's control functions can be provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the promoter is an inducible promoter, e.g., a promoter 5 regulated by a steroid hormone, by a polypeptide hormone (e.g., by means of a signal transduction pathway), or by a heterologous polypeptide (e.g., the tetracycline-inducible systems, "Tet-On" and "Tet-Off"; see, e.g., Clontech Inc., CA, Gossen and Bujard (1992) *Proc. Natl. Acad. Sci. USA* 89:5547, and Paillard (1989) *Human Gene Therapy* 9:983).

In another embodiment, the recombinant mammalian expression vector is capable of 10 directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore 15 (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 20 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA 25 molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or 30 attenuated virus.

Another aspect the invention provides a host cell which includes a nucleic acid molecule described herein, e.g., a 33521 nucleic acid molecule within a recombinant expression vector or a 33521 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" 5 and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

10 A host cell can be any prokaryotic or eukaryotic cell. For example, a 33521 protein can be expressed in bacterial cells (such as *E. coli*), insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells (African green monkey kidney cells CV-1 origin SV40 cells; Gluzman (1981) *Cell*23:175-182)). Other suitable host cells are known to those skilled in the art.

15 Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation.

20 A host cell of the invention can be used to produce (i.e., express) a 33521 protein. Accordingly, the invention further provides methods for producing a 33521 protein using the host cells of the invention. In one embodiment, the method includes culturing the host cell of the invention (into which a recombinant expression vector encoding a 33521 protein has been introduced) in a suitable medium such that a 33521 protein is produced. In another 25 embodiment, the method further includes isolating a 33521 protein from the medium or the host cell.

In another aspect, the invention features, a cell or purified preparation of cells which include a 33521 transgene, or which otherwise misexpress 33521. The cell preparation can consist of human or non-human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, 30 or pig cells. In preferred embodiments, the cell or cells include a 33521 transgene, e.g., a heterologous form of a 33521, e.g., a gene derived from humans (in the case of a non-human

cell). The 33521 transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene that mis-expresses an endogenous 33521, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders that are related to mutated or mis-expressed 5 33521 alleles or for use in drug screening.

In another aspect, the invention features, a human cell, e.g., a hematopoietic stem cell, transformed with nucleic acid which encodes a subject 33521 polypeptide.

Also provided are cells, preferably human cells, e.g., human hematopoietic or fibroblast cells, in which an endogenous 33521 is under the control of a regulatory sequence 10 that does not normally control the expression of the endogenous 33521 gene. The expression characteristics of an endogenous gene within a cell, e.g., a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the endogenous 33521 gene. For example, an endogenous 33521 gene which is "transcriptionally silent," e.g., not 15 normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, e.g., Chappel, US 5,272,071; WO 91/06667, published in May 16, 1991.

20 In a preferred embodiment, recombinant cells described herein can be used for replacement therapy in a subject. For example, a nucleic acid encoding a 33521 polypeptide operably linked to an inducible promoter (e.g., a steroid hormone receptor-regulated promoter) is introduced into a human or nonhuman, e.g., mammalian, e.g., porcine recombinant cell. The cell is cultivated and encapsulated in a biocompatible material, such 25 as poly-lysine alginate, and subsequently implanted into the subject. See, e.g., Lanza (1996) *Nat. Biotechnol.* 14:1107; Joki *et al.* (2001) *Nat. Biotechnol.* 19:35; and U.S. Patent No. 5,876,742. Production of 33521 polypeptide can be regulated in the subject by administering an agent (e.g., a steroid hormone) to the subject. In another preferred embodiment, the 30 implanted recombinant cells express and secrete an antibody specific for a 33521 polypeptide. The antibody can be any antibody or any antibody derivative described herein.

Transgenic Animals

The invention provides non-human transgenic animals. Such animals are useful for studying the function and/or activity of a 33521 protein and for identifying and/or evaluating modulators of 33521 activity. As used herein, a "transgenic animal" is a non-human animal, 5 preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A 10 transgene is exogenous DNA or a rearrangement, e.g., a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, other transgenes, e.g., a knockout, reduce expression. Thus, a transgenic animal can be one in which an endogenous 33521 15 gene has been altered by, e.g., by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene of the invention to direct expression of a 33521 protein to particular cells. A transgenic founder animal can be identified based upon 20 the presence of a 33521 transgene in its genome and/or expression of 33521 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 33521 protein can further be bred to other transgenic animals carrying other transgenes.

33521 proteins or polypeptides can be expressed in transgenic animals or plants, e.g., 25 a nucleic acid encoding the protein or polypeptide can be introduced into the genome of an animal. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, e.g., a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Suitable animals are mice, pigs, cows, goats, and sheep.

The invention also includes a population of cells from a transgenic animal, as 30 discussed, e.g., below.

Uses

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and 5 pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

The isolated nucleic acid molecules of the invention can be used, for example, to express a 33521 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect a 33521 mRNA (e.g., in a biological sample) or a genetic alteration in a 33521 gene, and to modulate 33521 activity, as described further below. The 10 33521 proteins can be used to treat disorders characterized by insufficient or excessive production of a 33521 substrate or production of 33521 inhibitors. In addition, the 33521 proteins can be used to screen for naturally occurring 33521 substrates, to screen for drugs or compounds which modulate 33521 activity, as well as to treat disorders characterized by 15 insufficient or excessive production of 33521 protein or production of 33521 protein forms which have decreased, aberrant or unwanted activity compared to 33521 wild type protein (e.g., aberrant cell proliferation and/or differentiation). Moreover, the anti-33521 antibodies of the invention can be used to detect and isolate 33521 proteins, regulate the bioavailability 20 of 33521 proteins, and modulate 33521 activity.

A method of evaluating a compound for the ability to interact with, e.g., bind, a 25 subject 33521 polypeptide is provided. The method includes: contacting the compound with the subject 33521 polypeptide; and evaluating ability of the compound to interact with, e.g., to bind or form a complex with the subject 33521 polypeptide. This method can be performed in vitro, e.g., in a cell free system, or in vivo, e.g., in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules that interact with 25 subject 33521 polypeptide. It can also be used to find natural or synthetic inhibitors of subject 33521 polypeptide. Screening methods are discussed in more detail below.

Screening Assays

The invention provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., proteins, peptides, 30 peptidomimetics, peptoids, small molecules or other drugs) which bind to 33521 proteins,

have a stimulatory or inhibitory effect on, for example, 33521 expression or 33521 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 33521 substrate. Compounds thus identified can be used to modulate the activity of target gene products (e.g., 33521 genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 33521 protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds that bind to or modulate an activity of a 33521 protein or polypeptide or a biologically active portion thereof.

In one embodiment, an activity of a 33521 protein can be assayed, e.g., assayed for nucleotide exchange activity toward Rho proteins. An example of such an "exchange assay" can be found, for example, in Fleming et al., *J. Biol Chem.* 274: 12753-12758 (1999), wherein the nucleotide exchange activity of TIAM1 toward Rac1 was assayed. Briefly, the activity of TIAM1 was observed by incubating [³H]GDP-Rac1 with TIAM1 for a specified time period, and then measuring the amount of [³H]GDP remaining bound to Rac1 following the incubation period.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, e.g., Zuckermann, R.N. et al. (1994) *J. Med. Chem.* 37:2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb et al. (1994)

Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann *et al.* (1994) *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

5 Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner U.S. Patent No. 5,223,409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 10 249:404-406; Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382; Felici (1991) *J. Mol. Biol.* 222:301-310; Ladner *supra*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a 15 33521 protein or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate 33521 activity is determined. Determining the ability of the test compound to modulate 33521 activity can be accomplished by monitoring, for example, stimulation of guanine nucleotide exchange on a Rho protein. The cell, for example, can be of mammalian origin, e.g., human.

The ability of the test compound to modulate 33521 binding to a compound, e.g., a 20 33521 substrate, or to bind to 33521 can also be evaluated. This can be accomplished, for example, by coupling the compound, e.g., the substrate, with a radioisotope or enzymatic 25 label such that binding of the compound, e.g., the substrate, to 33521 can be determined by detecting the labeled compound, e.g., substrate, in a complex. Alternatively, 33521 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 33521 binding to a 33521 substrate in a complex. For example, compounds (e.g., 33521 substrates) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

30 The ability of a compound (e.g., a 33521 substrate) to interact with 33521 with or without the labeling of any of the interactants can be evaluated. For example, a

microphysiometer can be used to detect the interaction of a compound with 33521 without the labeling of either the compound or the 33521. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 33521.

In yet another embodiment, a cell-free assay is provided in which a 33521 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 33521 protein or biologically active portion thereof is evaluated.

Preferred biologically active portions of the 33521 proteins to be used in assays of the present invention include fragments which participate in interactions with non-33521 molecules, e.g., fragments with high surface probability scores.

Soluble and/or membrane-bound forms of isolated proteins (e.g., 33521 proteins or biologically active portions thereof) can be used in the cell-free assays of the invention.

When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltofside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)_n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

The interaction between two molecules can also be detected, e.g., using fluorescence energy transfer (FET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos, *et al.*, U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light,

such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' 5 molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

In another embodiment, determining the ability of the 33521 protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis 10 (BIA) (see, e.g., Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (e.g., BIACore). Changes in the mass at the binding surface (indicative of a binding event) 15 result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

In one embodiment, the target gene product or the test substance is anchored onto a solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be 20 anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

It may be desirable to immobilize either 33521, an anti-33521 antibody or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to 25 a 33521 protein, or interaction of a 33521 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S- 30 transferase/33521 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione

derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 33521 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 33521 binding or activity determined using standard techniques.

Other techniques for immobilizing either a 33521 protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated 33521 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

In one embodiment, this assay is performed utilizing antibodies reactive with 33521 protein or target molecules but which do not interfere with binding of the 33521 protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or 33521 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 33521 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 33521 protein or target molecule.

Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A.P., (1993) *Trends Biochem Sci* 18:284-7);

5 chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, e.g., Ausubel, F. et al., eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. et al., eds. (1999) *Current Protocols in Molecular Biology*, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, e.g., Heegaard, N.H.,

10 (1998) *J Mol Recognit* 11:141-8; Hage, D.S., and Tweed, S.A. (1997) *J Chromatogr B Biomed Sci Appl.* 699:499-525). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

In a preferred embodiment, the assay includes contacting the 33521 protein or biologically active portion thereof with a known compound which binds 33521 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 33521 protein, wherein determining the ability of the test compound to interact with a 33521 protein includes determining the ability of the test compound to preferentially bind to 33521 or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

The target gene products of the invention can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity 25 of the target gene product. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and small molecules. The preferred target genes/products for use in this embodiment are the 33521 genes herein identified. In an alternative embodiment, the invention provides methods for determining the ability of the test compound to modulate the activity of a 33521 protein through modulation of the activity of a downstream effector of 30 a 33521 target molecule. For example, the activity of the effector molecule on an appropriate

target can be determined, or the binding of the effector to an appropriate target can be determined, as previously described.

To identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner(s), a reaction mixture containing the target gene product and the binding partner is prepared, under conditions and for a time sufficient, to allow the two products to form complex. In order to test an inhibitory agent, the reaction mixture is provided in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of the target gene and its cellular or extracellular binding partner.

Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene product and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene product and the interactive binding partner.

Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and mutant target gene product. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal target gene products.

These assays can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the target gene product or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the target gene products and the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partner, is anchored onto a solid surface (e.g., a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments. Alternatively, an 5 immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain 10 immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect 15 complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, 20 and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

25 In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared in that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 that utilizes this approach for 30 immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above

background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

In yet another aspect, the 33521 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) 5 *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 33521 ("33521-binding proteins" or "33521-bp") and are involved in 33521 activity. Such 33521-bps can be activators or inhibitors of signals by the 33521 proteins or 33521 targets as, for example, 10 downstream elements of a 33521-mediated signaling pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 33521 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., 15 GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. (Alternatively the 33521 protein can be fused to the activator domain.) If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a 33521-dependent complex, the DNA-binding and activation domains of the 20 transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., lacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 33521 protein.

25 In another embodiment, modulators of 33521 expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of 33521 mRNA or protein evaluated relative to the level of expression of 33521 mRNA or protein in the absence of the candidate compound. When expression of 33521 mRNA or protein is greater in the presence of the candidate compound than in its absence, the 30 candidate compound is identified as a stimulator of 33521 mRNA or protein expression. Alternatively, when expression of 33521 mRNA or protein is less (statistically significantly

less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 33521 mRNA or protein expression. The level of 33521 mRNA or protein expression can be determined by methods described herein for detecting 33521 mRNA or protein.

5 In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a 33521 protein can be confirmed *in vivo*, e.g., in an animal such as an animal model for disorders of cell proliferation and differentiation, e.g., cancer and metastasis.

10 This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein (e.g., a 33521 modulating agent, an antisense 33521 nucleic acid molecule, a 33521-specific antibody, or a 33521-binding partner) in an appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of
15 treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be used for treatments as described herein.

Detection Assays

Portions or fragments of the nucleic acid sequences identified herein can be used as polynucleotide reagents. For example, these sequences can be used to: (i) map their
20 respective genes on a chromosome e.g., to locate gene regions associated with genetic disease or to associate 33521 with a disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

Chromosome Mapping

25 The 33521 nucleotide sequences or portions thereof can be used to map the location of the 33521 genes on a chromosome. This process is called chromosome mapping. Chromosome mapping is useful in correlating the 33521 sequences with genes associated with disease.

Briefly, 33521 genes can be mapped to chromosomes by preparing PCR primers
30 (preferably 15-25 bp in length) from the 33521 nucleotide sequences. These primers can

then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the 33521 sequences will yield an amplified fragment.

A panel of somatic cell hybrids in which each cell line contains either a single human 5 chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, can allow easy mapping of individual genes to specific human chromosomes. (DEustachio P. *et al.* (1983) *Science* 220:919-924).

Other mapping strategies e.g., *in situ* hybridization (described in Fan, Y. *et al.* (1990) 10 *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries can be used to map 33521 to a chromosomal location.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase 15 chromosomal spread can further be used to provide a precise chromosomal location in one step. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique 20 chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual of Basic Techniques* ((1988) Pergamon Press, New York).

Reagents for chromosome mapping can be used individually to mark a single 25 chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical 30 position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available online through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through

linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. et al. (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the 33521 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

33521 sequences can be used to identify individuals from biological samples using, e.g., restriction fragment length polymorphism (RFLP). In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, the fragments separated, e.g., in a Southern blot, and probed to yield bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the 33521 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it. Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences.

Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can provide positive individual identification with a

panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from 33521 nucleotide sequences described herein is used to 5 generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

Use of Partial 33521 Sequences in Forensic Biology

10 DNA-based identification techniques can also be used in forensic biology. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

15 The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for 20 identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 (e.g., fragments derived from the noncoding regions of SEQ ID NO:1 having a length of at least 20 bases, preferably at least 30 bases) are particularly appropriate for this use.

The 33521 nucleotide sequences described herein can further be used to provide 25 polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 33521 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., 33521 primers or probes can be used to 30 screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual.

5 Generally, the invention provides, a method of determining if a subject is at risk for a disorder related to a lesion in or the misexpression of a gene which encodes 33521.

Such disorders include, e.g., a disorder associated with the misexpression of 33521 gene; a disorder of cell proliferation, e.g., cancer, or a disorder of cell motility, e.g., metastatic cancer.

10 The method includes one or more of the following:

detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of the 33521 gene, or detecting the presence or absence of a mutation in a region which controls the expression of the gene, e.g., a mutation in the 5' control region;

15 detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure of the 33521 gene;

detecting, in a tissue of the subject, the misexpression of the 33521 gene, at the mRNA level, e.g., detecting a non-wild type level of a mRNA ;

detecting, in a tissue of the subject, the misexpression of the gene, at the protein level, e.g., detecting a non-wild type level of a 33521 polypeptide.

20 In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from the 33521 gene; an insertion of one or more nucleotides into the gene, a point mutation, e.g., a substitution of one or more nucleotides of the gene, a gross chromosomal rearrangement of the gene, e.g., a translocation, inversion, or deletion.

25 For example, detecting the genetic lesion can include: (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence from SEQ ID NO:1, or naturally occurring mutants thereof or 5' or 3' flanking sequences naturally associated with the 33521 gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, e.g., *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

In preferred embodiments detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of the 33521 gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of 33521.

5 Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

In preferred embodiments the method includes determining the structure of a 33521 gene, an abnormal structure being indicative of risk for the disorder.

10 In preferred embodiments the method includes contacting a sample from the subject with an antibody to the 33521 protein or a nucleic acid, which hybridizes specifically with the gene. These and other embodiments are discussed below.

Diagnostic and Prognostic Assays

15 Diagnostic and prognostic assays of the invention include method for assessing the expression level of 33521 molecules and for identifying variations and mutations in the sequence of 33521 molecules.

Expression Monitoring and Profiling

The presence, level, or absence of 33521 protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 33521 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes 33521 protein such that the presence of 33521 protein or nucleic acid is detected in the biological sample. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is serum. The level of expression of the 33521 gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the 33521 genes; measuring the amount of protein encoded by the 33521 genes; or measuring the activity of the protein encoded by the 33521 genes.

30 The level of mRNA corresponding to the 33521 gene in a cell can be determined both by *in situ* and by *in vitro* formats.

The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the 5 mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length 33521 nucleic acid, such as the nucleic acid of SEQ ID NO:1, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 33521 mRNA or genomic DNA. The probe can be disposed on an address of an array, e.g., an array described below. 10 Other suitable probes for use in the diagnostic assays are described herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, 15 for example, in a two-dimensional gene chip array described below. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the 33521 genes.

The level of mRNA in a sample that is encoded by one of 33521 can be evaluated with nucleic acid amplification, e.g., by rtPCR (Mullis (1987) U.S. Patent No. 4,683,202), 20 ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.*, (1989), *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, U.S. Patent No. 5,854,033) or any other nucleic acid 25 amplification method, followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 30 nucleotides in length. Under appropriate conditions and with appropriate reagents, such

primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can 5 hybridize to mRNA that encodes the 33521 gene being analyzed.

In another embodiment, the methods further contacting a control sample with a compound or agent capable of detecting 33521 mRNA, or genomic DNA, and comparing the presence of 33521 mRNA or genomic DNA in the control sample with the presence of 33521 mRNA or genomic DNA in the test sample. In still another embodiment, serial analysis of 10 gene expression, as described in U.S. Patent No. 5,695,937, is used to detect 33521 transcript levels.

A variety of methods can be used to determine the level of protein encoded by 33521. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody with a sample, to evaluate the level of protein in the sample. In a 15 preferred embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or 20 antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

The detection methods can be used to detect 33521 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of 33521 protein include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, 25 enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo* techniques for detection of 33521 protein include introducing into a subject a labeled anti-33521 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. In another embodiment, the sample is labeled, e.g., biotinylated and then contacted to the 30 antibody, e.g., an anti-33521 antibody positioned on an antibody array (as described below). The sample can be detected, e.g., with avidin coupled to a fluorescent label.

In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting 33521 protein, and comparing the presence of 33521 protein in the control sample with the presence of 33521 protein in the test sample.

The invention also includes kits for detecting the presence of 33521 in a biological sample. For example, the kit can include a compound or agent capable of detecting 33521 protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 33521 protein or nucleic acid.

For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also include a buffering agent, a preservative, or a protein stabilizing agent. The kit can also include components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with misexpressed or aberrant or unwanted 33521 expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as pain or deregulated cell proliferation.

In one embodiment, a disease or disorder associated with aberrant or unwanted 33521 expression or activity is identified. A test sample is obtained from a subject and 33521 protein or nucleic acid (e.g., mRNA or genomic DNA) is evaluated, wherein the level, e.g., the presence or absence, of 33521 protein or nucleic acid is diagnostic for a subject having or

at risk of developing a disease or disorder associated with aberrant or unwanted 33521 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest, including a biological fluid (e.g., serum), cell sample, or tissue.

The prognostic assays described herein can be used to determine whether a subject
5 can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 33521 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a cell proliferation and differentiation disorder, e.g., cancer and metastasis.

10 In another aspect, the invention features a computer medium having a plurality of digitally encoded data records. Each data record includes a value representing the level of expression of 33521 in a sample, and a descriptor of the sample. The descriptor of the sample can be an identifier of the sample, a subject from which the sample was derived (e.g., a patient), a diagnosis, or a treatment (e.g., a preferred treatment). In a preferred
15 embodiment, the data record further includes values representing the level of expression of genes other than 33521 (e.g., other genes associated with a 33521-disorder, or other genes on an array). The data record can be structured as a table, e.g., a table that is part of a database such as a relational database (e.g., a SQL database of the Oracle or Sybase database environments).

20 Also featured is a method of evaluating a sample. The method includes providing a sample, e.g., from the subject, and determining a gene expression profile of the sample, wherein the profile includes a value representing the level of 33521 expression. The method can further include comparing the value or the profile (i.e., multiple values) to a reference value or reference profile. The gene expression profile of the sample can be obtained by any
25 of the methods described herein (e.g., by providing a nucleic acid from the sample and contacting the nucleic acid to an array). The method can be used to diagnose a disorder in a subject wherein a change in 33521 expression is an indication that the subject has or is disposed to having a disorder. The method can be used to monitor a treatment for a disorder in a subject. For example, the gene expression profile can be determined for a sample from a
30 subject undergoing treatment. The profile can be compared to a reference profile or to a

profile obtained from the subject prior to treatment or prior to onset of the disorder (see, e.g., Golub *et al.* (1999) *Science* 286:531).

In yet another aspect, the invention features a method of evaluating a test compound (see also, "Screening Assays", above). The method includes providing a cell and a test compound; contacting the test compound to the cell; obtaining a subject expression profile for the contacted cell; and comparing the subject expression profile to one or more reference profiles. The profiles include a value representing the level of 33521 expression. In a preferred embodiment, the subject expression profile is compared to a target profile, e.g., a profile for a normal cell or for desired condition of a cell. The test compound is evaluated favorably if the subject expression profile is more similar to the target profile than an expression profile obtained from an uncontacted cell.

In another aspect, the invention features, a method of evaluating a subject. The method includes: a) obtaining a sample from a subject, e.g., from a caregiver, e.g., a caregiver who obtains the sample from the subject; b) determining a subject expression profile for the sample. Optionally, the method further includes either or both of steps: c) comparing the subject expression profile to one or more reference expression profiles; and d) selecting the reference profile most similar to the subject reference profile. The subject expression profile and the reference profiles include a value representing the level of 33521 expression. A variety of routine statistical measures can be used to compare two reference profiles. One possible metric is the length of the distance vector that is the difference between the two profiles. Each of the subject and reference profile is represented as a multi-dimensional vector, wherein each dimension is a value in the profile.

The method can further include transmitting a result to a caregiver. The result can be the subject expression profile, a result of a comparison of the subject expression profile with another profile, a most similar reference profile, or a descriptor of any of the aforementioned. The result can be transmitted across a computer network, e.g., the result can be in the form of a computer transmission, e.g., a computer data signal embedded in a carrier wave.

Also featured is a computer medium having executable code for effecting the following steps: receive a subject expression profile; access a database of reference expression profiles; and either i) select a matching reference profile most similar to the subject expression profile or ii) determine at least one comparison score for the similarity of

the subject expression profile to at least one reference profile. The subject expression profile, and the reference expression profiles each include a value representing the level of 33521 expression.

Arrays and Uses Thereof

5 In another aspect, the invention features an array that includes a substrate having a plurality of addresses. At least one address of the plurality includes a capture probe that binds specifically to a 33521 molecule (e.g., a 33521 nucleic acid or a 33521 polypeptide). The array can have a density of at least than 10, 50, 100, 200, 500, 1,000, 2,000, or 10,000 or more addresses/cm², and ranges between. In a preferred embodiment, the plurality of 10 addresses includes at least 10, 100, 500, 1,000, 5,000, 10,000, 50,000 addresses. In a preferred embodiment, the plurality of addresses includes equal to or less than 10, 100, 500, 1,000, 5,000, 10,000, or 50,000 addresses. The substrate can be a two-dimensional substrate such as a glass slide, a wafer (e.g., silica or plastic), a mass spectroscopy plate, or a three-dimensional substrate such as a gel pad. Addresses in addition to address of the plurality can 15 be disposed on the array.

In a preferred embodiment, at least one address of the plurality includes a nucleic acid capture probe that hybridizes specifically to a 33521 nucleic acid, e.g., the sense or anti-sense strand. In one preferred embodiment, a subset of addresses of the plurality of addresses has a nucleic acid capture probe for 33521. Each address of the subset can include a capture probe 20 that hybridizes to a different region of a 33521 nucleic acid. In another preferred embodiment, addresses of the subset include a capture probe for a 33521 nucleic acid. Each address of the subset is unique, overlapping, and complementary to a different variant of 33521 (e.g., an allelic variant, or all possible hypothetical variants). The array can be used to sequence 33521 by hybridization (see, e.g., U.S. Patent No. 5,695,940).

25 An array can be generated by various methods, e.g., by photolithographic methods (see, e.g., U.S. Patent Nos. 5,143,854; 5,510,270; and 5,527,681), mechanical methods (e.g., directed-flow methods as described in U.S. Patent No. 5,384,261), pin-based methods (e.g., as described in U.S. Pat. No. 5,288,514), and bead-based techniques (e.g., as described in PCT US/93/04145).

30 In another preferred embodiment, at least one address of the plurality includes a polypeptide capture probe that binds specifically to a 33521 polypeptide or fragment thereof.

The polypeptide can be a naturally-occurring interaction partner of 33521 polypeptide. Preferably, the polypeptide is an antibody, e.g., an antibody described herein (see "Anti-33521 Antibodies," above), such as a monoclonal antibody or a single-chain antibody.

In another aspect, the invention features a method of analyzing the expression of 33521. The method includes providing an array as described above; contacting the array with a sample and detecting binding of a 33521-molecule (e.g., nucleic acid or polypeptide) to the array. In a preferred embodiment, the array is a nucleic acid array. Optionally the method further includes amplifying nucleic acid from the sample prior or during contact with the array.

In another embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array, particularly the expression of 33521. If a sufficient number of diverse samples is analyzed, clustering (e.g., hierarchical clustering, k-means clustering, Bayesian clustering and the like) can be used to identify other genes which are co-regulated with 33521. For example, the array can be used for the quantitation of the expression of multiple genes. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertained. Quantitative data can be used to group (e.g., cluster) genes on the basis of their tissue expression *per se* and level of expression in that tissue.

For example, array analysis of gene expression can be used to assess the effect of cell-cell interactions on 33521 expression. A first tissue can be perturbed and nucleic acid from a second tissue that interacts with the first tissue can be analyzed. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined, e.g., to monitor the effect of cell-cell interaction at the level of gene expression.

In another embodiment, cells are contacted with a therapeutic agent. The expression profile of the cells is determined using the array, and the expression profile is compared to the profile of like cells not contacted with the agent. For example, the assay can be used to determine or analyze the molecular basis of an undesirable effect of the therapeutic agent. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable

biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor expression of one or more genes in the array with respect to time. For example, samples obtained from different time points can be probed with the array. Such analysis can identify and/or characterize the development of a 33521-associated disease or disorder; and processes, such as a cellular transformation associated with a 33521-associated disease or disorder. The method can also evaluate the treatment and/or progression of a 33521-associated disease or disorder

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (e.g., including 33521) that could serve as a molecular target for diagnosis or therapeutic intervention.

In another aspect, the invention features an array having a plurality of addresses. Each address of the plurality includes a unique polypeptide. At least one address of the plurality has disposed thereon a 33521 polypeptide or fragment thereof. Methods of producing polypeptide arrays are described in the art, e.g., in De Wildt *et al.* (2000). *Nature Biotech.* 18, 989-994; Lueking *et al.* (1999). *Anal. Biochem.* 270, 103-111; Ge, H. (2000). *Nucleic Acids Res.* 28, e3, I-VII; MacBeath, G., and Schreiber, S.L. (2000). *Science* 289, 1760-1763; and WO 99/51773A1. In a preferred embodiment, each addresses of the plurality has disposed thereon a polypeptide at least 60, 70, 80, 85, 90, 95 or 99 % identical to a 33521 polypeptide or fragment thereof. For example, multiple variants of a 33521 polypeptide (e.g., encoded by allelic variants, site-directed mutants, random mutants, or combinatorial mutants) can be disposed at individual addresses of the plurality. Addresses in addition to the address of the plurality can be disposed on the array.

The polypeptide array can be used to detect a 33521 binding compound, e.g., an antibody in a sample from a subject with specificity for a 33521 polypeptide or the presence of a 33521-binding protein or ligand.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (e.g., ascertaining the effect of 33521 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

In another aspect, the invention features a method of analyzing a plurality of probes. The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express 33521 or from a cell or subject in which a 33521 mediated response has been elicited, e.g., by contact of the cell with 33521 nucleic acid or protein, or administration to the cell or subject 33521 nucleic acid or protein; providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express 33521 (or does not express as highly as in the case of the 33521 positive plurality of capture probes) or from a cell or subject which in which a 33521 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than a 33521 nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

In another aspect, the invention features a method of analyzing a plurality of probes or a sample. The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, contacting the array with a first sample from a cell or subject which express or mis-express 33521 or from a cell or subject in which a 33521-mediated response has been elicited, e.g., by contact of the cell with 33521 nucleic acid or protein, or administration to the cell or subject 33521 nucleic acid or protein; providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, and contacting the array with a second sample from a cell or subject which does not express 33521 (or does not express as highly as in the case of the 33521 positive plurality of capture probes) or from a cell or subject which in which a

33521 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); and comparing the binding of the first sample with the binding of the second sample. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody. The same array can be used for both samples or different arrays can be used. If different arrays are used the plurality of addresses with capture probes should be present on both arrays.

In another aspect, the invention features a method of analyzing 33521, e.g., analyzing structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing a 33521 nucleic acid or amino acid sequence; comparing the 33521 sequence with one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze 33521.

Detection of Variations or Mutations

The methods of the invention can also be used to detect genetic alterations in a 33521 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in 33521 protein activity or nucleic acid expression, such as a cell proliferation and differentiation disorder, e.g., cancer and metastasis. In preferred embodiments, the methods include detecting, in a sample from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 33521-protein, or the mis-expression of the 33521 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 33521 gene; 2) an addition of one or more nucleotides to a 33521 gene; 3) a substitution of one or more nucleotides of a 33521 gene, 4) a chromosomal rearrangement of a 33521 gene; 5) an alteration in the level of a messenger RNA transcript of a 33521 gene, 6) aberrant modification of a 33521 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 33521 gene, 8) a non-wild type level of a 33521-protein, 9) allelic loss of a 33521 gene, and 10) inappropriate post-translational modification of a 33521-protein.

An alteration can be detected without a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the

latter of which can be particularly useful for detecting point mutations in the 33521-gene. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 33521 gene under 5 conditions such that hybridization and amplification of the 33521-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein. Alternatively, 10 other amplification methods described herein or known in the art can be used.

In another embodiment, mutations in a 33521 gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined, e.g., by gel 15 electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in 33521 can be identified by hybridizing a 20 sample and control nucleic acids, e.g., DNA or RNA, two-dimensional arrays, e.g., chip based arrays. Such arrays include a plurality of addresses, each of which is positionally distinguishable from the other. A different probe is located at each address of the plurality. A probe can be complementary to a region of a 33521 nucleic acid or a putative variant (e.g., allelic variant) thereof. A probe can have one or more mismatches to a region of a 33521 25 nucleic acid (e.g., a destabilizing mismatch). The arrays can have a high density of addresses, e.g., can contain hundreds or thousands of oligonucleotides probes (Cronin, M.T. et al. (1996) *Human Mutation* 7: 244-255; Kozal, M.J. et al. (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in 33521 can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. et al. *supra*. Briefly, a 30 first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays

of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one 5 complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 33521 gene and detect mutations by comparing the sequence of the sample 33521 with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays 10 ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry.

Other methods for detecting mutations in the 33521 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242; Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295).

15 In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 33521 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T 20 at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662; U.S. Patent No. 5,459,039).

25 In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 33521 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 33521 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the 30 detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than

DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

5 In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by
10 PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). A further method of detecting point mutations is the chemical ligation of oligonucleotides as described in Xu *et al.* ((2001) *Nature Biotechnol.* 19:148). Adjacent oligonucleotides, one of which selectively anneals to the query site, are ligated together if the nucleotide at the query site of the sample nucleic acid is complementary to the query
20 oligonucleotide; ligation can be monitored, e.g., by fluorescent dyes coupled to the oligonucleotides.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA*

88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

In another aspect, the invention features a set of oligonucleotides. The set includes a 5 plurality of oligonucleotides, each of which is at least partially complementary (e.g., at least .50%, 60%, 70%, 80%, 90%, 92%, 95%, 97%, 98%, or 99% complementary) to a 33521 nucleic acid.

In a preferred embodiment the set includes a first and a second oligonucleotide. The first and second oligonucleotide can hybridize to the same or to different locations of SEQ ID 10 NO:1 or the complement of SEQ ID NO:1. Different locations can be different but overlapping, or non-overlapping on the same strand. The first and second oligonucleotide can hybridize to sites on the same or on different strands.

The set can be useful, e.g., for identifying SNP's, or identifying specific alleles of 15 33521. In a preferred embodiment, each oligonucleotide of the set has a different nucleotide at an interrogation position. In one embodiment, the set includes two oligonucleotides, each complementary to a different allele at a locus, e.g., a biallelic or polymorphic locus.

In another embodiment, the set includes four oligonucleotides, each having a different nucleotide (e.g., adenine, guanine, cytosine, or thymidine) at the interrogation position. The 20 interrogation position can be a SNP or the site of a mutation. In another preferred embodiment, the oligonucleotides of the plurality are identical in sequence to one another (except for differences in length). The oligonucleotides can be provided with differential labels, such that an oligonucleotide that hybridizes to one allele provides a signal that is distinguishable from an oligonucleotide that hybridizes to a second allele. In still another embodiment, at least one of the oligonucleotides of the set has a nucleotide change at a 25 position in addition to a query position, e.g., a destabilizing mutation to decrease the T_m of the oligonucleotide. In another embodiment, at least one oligonucleotide of the set has a non-natural nucleotide, e.g., inosine. In a preferred embodiment, the oligonucleotides are attached to a solid support, e.g., to different addresses of an array or to different beads or nanoparticles.

30 In a preferred embodiment the set of oligo nucleotides can be used to specifically amplify, e.g., by PCR, or detect, a 33521 nucleic acid.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a 33521 gene.

5 Use of 33521 Molecules as Surrogate Markers

The 33521 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the 10 33521 molecules of the invention may be detected, and may be correlated with one or more biological states *in vivo*. For example, the 33521 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the 15 progression of a disease or disorder (e.g., with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent 20 of a disease state or disorder is difficult to assess through standard methodologies (e.g., early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (e.g., an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of 25 surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The 33521 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a "pharmacodynamic marker" is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic 30 marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of

the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the 5 pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug in vivo. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since 10 even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., a 33521 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-33521 antibodies may be employed in an immune-based detection system for a 33521 protein 15 marker, or 33521-specific radiolabeled probes may be used to detect a 33521 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis 20 *et al.* (1991) *Env. Health Perspect.* 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

The 33521 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, e.g., McLeod *et al.* (1999) *Eur. J. Cancer* 35:1650-1652). The presence or quantity of the 25 pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., 33521 30 protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the

subject. Similarly, the presence or absence of a specific sequence mutation in 33521 DNA may correlate 33521 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

5 Pharmaceutical Compositions

The nucleic acid and polypeptides, fragments thereof, as well as anti-33521 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be

sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, 5 glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, 10 ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

15 Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the 20 case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the 25 purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of 30 the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating

agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit

containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the 5 LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such 10 compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no 15 toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound 20 which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 25 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The skilled artisan 30 will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder,

previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

5 For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be
10 used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank *et al.* ((1997) *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193).

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include,
15 but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic
20 compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order
30 to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at

first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, e.g., maytansinol (see US Patent No. 5,208,020), CC-1065 (see US Patent Nos. 5,475,092, 5,585,499, 5,846,545) and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, CC-1065, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine, vinblastine, taxol and maytansinoids). Radioactive ions include, but are not limited to iodine, yttrium and praseodymium.

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage

colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

5 The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in
10 an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

15 The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Methods of Treatment

20 The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted 33521 expression or activity. As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but
25 is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

30 With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term

refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 33521 molecules of the present invention or 33521 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 33521 expression or activity, by administering to the subject a 33521 or an agent which modulates 33521 expression or at least one 33521 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted 33521 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 33521 aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of 33521 aberrance, for example, a 33521, 33521 agonist or 33521 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

It is possible that some 33521 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of disorder symptoms.

Aberrant expression and/or activity of 33521 molecules may mediate disorders associated with bone metabolism. "Bone metabolism" refers to direct or indirect effects in the formation or degeneration of bone structures, e.g., bone formation, bone resorption, etc., which may ultimately affect the concentrations in serum of calcium and phosphate. This term also includes activities mediated by 33521 molecules effects in bone cells, e.g. osteoclasts and osteoblasts, that may in turn result in bone formation and degeneration. For example, 33521 molecules may support different activities of bone resorbing osteoclasts such as the stimulation of differentiation of monocytes and mononuclear phagocytes into

osteoclasts. Accordingly, 33521 molecules that modulate the production of bone cells can influence bone formation and degeneration, and thus may be used to treat bone disorders. Examples of such disorders include, but are not limited to, osteoporosis, osteodystrophy, osteomalacia, rickets, osteitis fibrosa cystica, renal osteodystrophy, osteosclerosis, anti-
5 convulsant treatment, osteopenia, fibrogenesis-imperfecta ossium, secondary hyperparathyroidism, hypoparathyroidism, hyperparathyroidism, cirrhosis, obstructive jaundice, drug induced metabolism, medullary carcinoma, chronic renal disease, rickets, sarcoidosis, glucocorticoid antagonism, malabsorption syndrome, steatorrhea, tropical sprue, idiopathic hypercalcemia and milk fever.

10 The 33521 nucleic acid and protein of the invention can be used to treat and/or diagnose a variety of immune disorders. Examples of immune disorders or diseases include, but are not limited to, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis,
15 acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host disease, cases of
20 transplantation, and allergy such as, atopic allergy.

25 Examples of disorders involving the heart or "cardiovascular disorder" include, but are not limited to, a disease, disorder, or state involving the cardiovascular system, e.g., the heart, the blood vessels, and/or the blood. A cardiovascular disorder can be caused by an imbalance in arterial pressure, a malfunction of the heart, or an occlusion of a blood vessel,
30 e.g., by a thrombus. Examples of such disorders include hypertension, atherosclerosis,

coronary artery spasm, congestive heart failure, coronary artery disease, valvular disease, arrhythmias, and cardiomyopathies.

Disorders which may be treated or diagnosed by methods described herein include, but are not limited to, disorders associated with an accumulation in the liver of fibrous tissue, such as that resulting from an imbalance between production and degradation of the extracellular matrix accompanied by the collapse and condensation of preexisting fibers. The methods described herein can be used to diagnose or treat hepatocellular necrosis or injury induced by a wide variety of agents including processes which disturb homeostasis, such as an inflammatory process, tissue damage resulting from toxic injury or altered hepatic blood flow, and infections (e.g., bacterial, viral and parasitic). For example, the methods can be used for the early detection of hepatic injury, such as portal hypertension or hepatic fibrosis. In addition, the methods can be employed to detect liver fibrosis attributed to inborn errors of metabolism, for example, fibrosis resulting from a storage disorder such as Gaucher's disease (lipid abnormalities) or a glycogen storage disease, A1-antitrypsin deficiency; a disorder mediating the accumulation (e.g., storage) of an exogenous substance, for example, hemochromatosis (iron-overload syndrome) and copper storage diseases (Wilson's disease), disorders resulting in the accumulation of a toxic metabolite (e.g., tyrosinemia, fructosemia and galactosemia) and peroxisomal disorders (e.g., Zellweger syndrome). Additionally, the methods described herein may be useful for the early detection and treatment of liver injury associated with the administration of various chemicals or drugs, such as for example, methotrexate, isoniazid, oxyphenisatin, methyldopa, chlorpromazine, tolbutamide or alcohol, or which represents a hepatic manifestation of a vascular disorder such as obstruction of either the intrahepatic or extrahepatic bile flow or an alteration in hepatic circulation resulting, for example, from chronic heart failure, veno-occlusive disease, portal vein thrombosis or Budd-Chiari syndrome.

Additionally, 33521 molecules may play an important role in the etiology of certain viral diseases, including but not limited to Hepatitis B, Hepatitis C and Herpes Simplex Virus (HSV). Modulators of 33521 activity could be used to control viral diseases. The modulators can be used in the treatment and/or diagnosis of viral infected tissue or virus-associated tissue fibrosis, especially liver and liver fibrosis. Also, 33521 modulators can be

used in the treatment and/or diagnosis of virus-associated carcinoma, especially hepatocellular cancer.

Additionally, 33521 may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, 5 anorexia nervosa, cachexia, lipid disorders, and diabetes. Examples of pain disorders include, but are not limited to, pain response elicited during various forms of tissue injury, e.g., inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L. (1987) *Pain*, New York:McGraw-Hill); pain associated with musculoskeletal disorders, e.g., joint pain; tooth pain; headaches; pain associated with 10 surgery; pain related to irritable bowel syndrome; or chest pain.

As discussed, successful treatment of 33521 disorders can be brought about by techniques that serve to inhibit the expression or activity of target gene products. For example, compounds, e.g., an agent identified using an assays described above, that proves to exhibit negative modulatory activity, can be used in accordance with the invention to prevent 15 and/or ameliorate symptoms of 33521 disorders. Such molecules can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, F(ab')₂ and Fab expression library fragments, scFV molecules, and epitope-binding fragments thereof).

Further, antisense and ribozyme molecules that inhibit expression of the target gene 20 can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above.

It is possible that the use of antisense, ribozyme, and/or triple helix molecules to 25 reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and 30 express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an

extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

Another method by which nucleic acid molecules may be utilized in treating or preventing a disease characterized by 33521 expression is through the use of aptamer molecules specific for 33521 protein. Aptamers are nucleic acid molecules having a tertiary structure which permits them to specifically bind to protein ligands (see, e.g., Osborne, *et al.* (1997) *Curr. Opin. Chem Biol.* 1: 5-9; and Patel, D.J. (1997) *Curr Opin Chem Biol* 1:32-46). Since nucleic acid molecules may in many cases be more conveniently introduced into target cells than therapeutic protein molecules may be, aptamers offer a method by which 33521 protein activity may be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

Antibodies can be generated that are both specific for target gene product and that reduce target gene product activity. Such antibodies may, therefore, be administered in instances whereby negative modulatory techniques are appropriate for the treatment of 33521 disorders. For a description of antibodies, see the Antibody section above.

In circumstances wherein injection of an animal or a human subject with a 33521 protein or epitope for stimulating antibody production is harmful to the subject, it is possible to generate an immune response against 33521 through the use of anti-idiotypic antibodies (see, for example, Herlyn, D. (1999) *Ann Med* 31:66-78; and Bhattacharya-Chatterjee, M., and Foon, K.A. (1998) *Cancer Treat Res.* 94:51-68). If an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-anti-idiotypic antibodies, which should be specific to the 33521 protein. Vaccines directed to a disease characterized by 33521 expression may also be generated in this fashion.

In instances where the target antigen is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding

single-chain antibodies within the target cell population (see e.g., Marasco *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

The identified compounds that inhibit target gene expression, synthesis and/or activity can be administered to a patient at therapeutically effective doses to prevent, treat or 5 ameliorate 33521 disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of the disorders. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures as described above.

The data obtained from the cell culture assays and animal studies can be used in 10 formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture 15 assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

20 Another example of determination of effective dose for an individual is the ability to directly assay levels of "free" and "bound" compound in the serum of the test subject. Such assays may utilize antibody mimics and/or "biosensors" that have been created through molecular imprinting techniques. The compound which is able to modulate 33521 activity is used as a template, or "imprinting molecule", to spatially organize polymerizable monomers prior to 25 their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated "negative image" of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell, R. J. *et al* (1996) *Current Opinion in Biotechnology* 7:89-94 and in Shea, K.J. (1994) *Trends in Polymer Science* 2:166-173. Such 30 "imprinted" affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted

matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, G. *et al.* (1993) *Nature* 361:645-647. Through the use of isotope-labeling, the "free" concentration of compound which modulates the expression or activity of 33521 can be readily monitored and used in calculations of IC₅₀.

5 Such "imprinted" affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC₅₀. An rudimentary example of such a "biosensor" is discussed in Kriz, D. *et al.*

10 (1995) *Analytical Chemistry* 67:2142-2144.

Another aspect of the invention pertains to methods of modulating 33521 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a 33521 or agent that modulates one or more of the activities of 33521 protein activity associated with the cell. An agent that modulates 33521 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 33521 protein (e.g., a 33521 substrate or receptor), a 33521 antibody, a 33521 agonist or antagonist, a peptidomimetic of a 33521 agonist or antagonist, or other small molecule.

In one embodiment, the agent stimulates one or 33521 activities. Examples of such 20 stimulatory agents include active 33521 protein and a nucleic acid molecule encoding 33521. In another embodiment, the agent inhibits one or more 33521 activities. Examples of such inhibitory agents include antisense 33521 nucleic acid molecules, anti-33521 antibodies, and 33521 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject).

25 As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 33521 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up regulates or down regulates) 33521 expression or activity. In 30 another embodiment, the method involves administering a 33521 protein or nucleic acid

molecule as therapy to compensate for reduced, aberrant, or unwanted 33521 expression or activity.

Stimulation of 33521 activity is desirable in situations in which 33521 is abnormally downregulated and/or in which increased 33521 activity is likely to have a beneficial effect.

5 For example, stimulation of 33521 activity is desirable in situations in which a 33521 is downregulated and/or in which increased 33521 activity is likely to have a beneficial effect. Likewise, inhibition of 33521 activity is desirable in situations in which 33521 is abnormally upregulated and/or in which decreased 33521 activity is likely to have a beneficial effect.

10 Pharmacogenomics

The 33521 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 33521 activity (e.g., 33521 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) 33521 associated disorders (e.g., cell proliferation and differentiation disorder, e.g., cancer and metastasis) associated with aberrant or unwanted 15 33521 activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood 20 concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 33521 molecule or 33521 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a 33521 molecule or 33521 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the 25 response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23:983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43:254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions 30 transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or

as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

5 One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be
10 compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in
15 a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be
20 tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach," can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (e.g., a 33521 protein of the present invention), all common variants 25 of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

Alternatively, a method termed the "gene expression profiling," can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a 33521 molecule or 33521 modulator of the present invention) can 30 give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance 5 therapeutic or prophylactic efficiency when treating a subject with a 33521 molecule or 33521 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more 10 of the gene products encoded by one or more of the 33521 genes of the present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the 33521 genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking 15 the activity of one or more of the resistance proteins, target cells, e.g., human cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

Monitoring the influence of agents (e.g., drugs) on the expression or activity of a 33521 protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 33521 gene expression, protein levels, or upregulate 33521 activity, can be monitored in clinical trials of subjects 20 exhibiting decreased 33521 gene expression, protein levels, or downregulated 33521 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease 33521 gene expression, protein levels, or downregulate 33521 activity, can be monitored in clinical trials of subjects exhibiting increased 33521 gene expression, protein levels, or upregulated 33521 activity. In such clinical trials, the expression or activity of a 33521 gene, 25 and preferably, other genes that have been implicated in, for example, a 33521-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

33521 Informatics

The sequence of a 33521 molecule is provided in a variety of media to facilitate use thereof. A sequence can be provided as a manufacture, other than an isolated nucleic acid or 30 amino acid molecule, which contains a 33521. Such a manufacture can provide a nucleotide or amino acid sequence, e.g., an open reading frame, in a form which allows examination of

the manufacture using means not directly applicable to examining the nucleotide or amino acid sequences, or a subset thereof, as they exists in nature or in purified form. The sequence information can include, but is not limited to, 33521 full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including 5 single nucleotide polymorphisms (SNPs), epitope sequence, and the like. In a preferred embodiment, the manufacture is a machine-readable medium, e.g., a magnetic, optical, chemical or mechanical information storage device.

As used herein, "machine-readable media" refers to any medium that can be read and accessed directly by a machine, e.g., a digital computer or analogue computer. Non-limiting 10 examples of a computer include a desktop PC, laptop, mainframe, server (e.g., a web server, network server, or server farm), handheld digital assistant, pager, mobile telephone, and the like. The computer can be stand-alone or connected to a communications network, e.g., a local area network (such as a VPN or intranet), a wide area network (e.g., an Extranet or the Internet), or a telephone network (e.g., a wireless, DSL, or ISDN network). Machine- 15 readable media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM, ROM, EPROM, EEPROM, flash memory, and the like; and hybrids of these categories such as magnetic/optical storage media.

A variety of data storage structures are available to a skilled artisan for creating a 20 machine-readable medium having recorded thereon a nucleotide or amino acid sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a 25 word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. The skilled artisan can readily adapt any number of data processor structuring formats (e.g., text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence 30 information of the present invention.

In a preferred embodiment, the sequence information is stored in a relational database (such as Sybase or Oracle). The database can have a first table for storing sequence (nucleic acid and/or amino acid sequence) information. The sequence information can be stored in one field (e.g., a first column) of a table row and an identifier for the sequence can be stored in another field (e.g., a second column) of the table row. The database can have a second table, e.g., storing annotations. The second table can have a field for the sequence identifier, a field for a descriptor or annotation text (e.g., the descriptor can refer to a functionality of the sequence, a field for the initial position in the sequence to which the annotation refers, and a field for the ultimate position in the sequence to which the annotation refers. Non-limiting examples for annotation to nucleic acid sequences include polymorphisms (e.g., SNP's) translational regulatory sites and splice junctions. Non-limiting examples for annotations to amino acid sequence include polypeptide domains, e.g., a domain described herein; active sites and other functional amino acids; and modification sites.

By providing the nucleotide or amino acid sequences of the invention in computer readable form, the skilled artisan can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid sequences of the invention in computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. A search is used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif. The search can be a BLAST search or other routine sequence comparison, e.g., a search described herein.

Thus, in one aspect, the invention features a method of analyzing 33521, e.g., analyzing structure, function, or relatedness to one or more other nucleic acid or amino acid sequences. The method includes: providing a 33521 nucleic acid or amino acid sequence; comparing the 33521 sequence with a second sequence, e.g., one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database to thereby analyze 33521. The method can be performed in a machine, e.g., a computer, or manually by a skilled artisan.

The method can include evaluating the sequence identity between a 33521 sequence and a database sequence. The method can be performed by accessing the database at a second site, e.g., over the Internet.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. Typical sequence lengths of a target sequence are from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium for analysis and comparison to other sequences. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software include, but are not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBI).

Thus, the invention features a method of making a computer readable record of a sequence of a 33521 sequence which includes recording the sequence on a computer readable matrix. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

In another aspect, the invention features, a method of analyzing a sequence. The method includes: providing a 33521 sequence, or record, in machine-readable form; comparing a second sequence to the 33521 sequence; thereby analyzing a sequence. Comparison can include comparing to sequences for sequence identity or determining if one sequence is included within the other, e.g., determining if the 33521 sequence includes a sequence being compared. In a preferred embodiment the 33521 or second sequence is stored on a first computer, e.g., at a first site and the comparison is performed, read, or recorded on a second computer, e.g., at a second site. E.g., the 33521 or second sequence can be stored in a public or proprietary database in one computer, and the results of the comparison performed, read, or recorded on a second computer. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the

transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

In another aspect, the invention provides a machine-readable medium for holding instructions for performing a method for determining whether a subject has a 33521-associated disease or disorder or a pre-disposition to a 33521-associated disease or disorder, wherein the method comprises the steps of determining 33521 sequence information associated with the subject and based on the 33521 sequence information, determining whether the subject has a 33521-associated disease or disorder or a pre-disposition to a 33521-associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

The invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a 33521-associated disease or disorder or a pre-disposition to a disease associated with a 33521 wherein the method comprises the steps of determining 33521 sequence information associated with the subject, and based on the 33521 sequence information, determining whether the subject has a 33521-associated disease or disorder or a pre-disposition to a 33521-associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. In a preferred embodiment, the method further includes the step of receiving information, e.g., phenotypic or genotypic information, associated with the subject and/or acquiring from a network phenotypic information associated with the subject. The information can be stored in a database, e.g., a relational database. In another embodiment, the method further includes accessing the database, e.g., for records relating to other subjects, comparing the 33521 sequence of the subject to the 33521 sequences in the database to thereby determine whether the subject as a 33521-associated disease or disorder, or a pre-disposition for such.

The present invention also provides in a network, a method for determining whether a subject has a 33521 associated disease or disorder or a pre-disposition to a 33521-associated disease or disorder associated with 33521, said method comprising the steps of receiving 33521 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to 33521 and/or corresponding to a 33521-associated disease or disorder (e.g., cell proliferation and differentiation disorder, e.g., cancer and metastasis), and based on one

or more of the phenotypic information, the 33521 information (e.g., sequence information and/or information related thereto), and the acquired information, determining whether the subject has a 33521-associated disease or disorder or a pre-disposition to a 33521-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a method for determining whether a subject has a 33521 -associated disease or disorder or a pre-disposition to a 33521-associated disease or disorder, said method comprising the steps of receiving information related to 33521 (e.g., sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to 33521 and/or related to a 33521-associated disease or disorder, and based on one or more of the phenotypic information, the 33521 information, and the acquired information, determining whether the subject has a 33521-associated disease or disorder or a pre-disposition to a 33521-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

This invention is further illustrated by the following examples that should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

20. Example 1: Identification and Characterization of Human 33521 cDNA

The human 33521 nucleic acid sequence is recited as follows:

GCACGTGACAAGCTGCACGCTCTAGAGTCGACCCAGCAGGCTCTGTGTATGAATGACAA
GGATACCTTCAGCCAGCTCATCTGGATGAATGAATGATTACACTAAGTGTCCCTCACAT
TCCTCTGTGGGCTCACTTCATGGACTCACTTGCGTGCTTGTAAATGTGCTGCGTTGCT
25 CCCAAGACCATGTAAAGCCTACTGACCACTAACCTCCCTCACAGCAGAAACTAGACGTCA
GGTTAAAATGGGCAACTCCGACAGTCAGTACACCCCTCAAGGATCTAAAATCATAGCAA
TACTATTACTGGTGCTAAGCAAATTCCCTGCTCCCTGAAAATACGTGGCGTTCATGCAA
AGAGGAAAAGTCATTGCATGGATGGGTCACGGGAGCAACGGAGCAGGTTACAAGTCCAG
30 GTCCCTGGCCCGAAGCTGCCTTCTCACTTTAAGAGTAACCAGCCTACGCATCGAGACT
CGGTGGCCCCACATGCAAGGTCTCCAGAGGTGTTGCCTACTCCACGCACAGGACAAATGC

CCCAGGGAAGGATTCCAGGGCATCAGTGCTGCTTCTCAACTGAGAATGGCTCCATTCT
 TGTTGGCACGAGCTGGCAGATAACCACATCACCTCCAGAGACTGCAACGGACACCTTCT
 CAACTGCTACGGGAGGAATGAGAGCATGCCCTCACCCCCACCGGGCGAAGACCGCAAGAG
 CCCCCGAGTGCTCATCAAAACGCTGGGAAGCCGGATGGGTTTAAGGGTCAGTTCCA
 5 CAATGGTGGCAACCCCAGCAAAGTGCCTGCAGAGGAUTGCAGTGAGCCGGTGCAGCTGCT
 GAGGTACTCACCTACCTTAGCATGGAAACCTCCCCTGTGCCCTGAAGCCAGGAGGGGTC
 CAGCGCCGATTCCCTGCCAGCCATGCCCTCTCCCACGGACTCTCGCCTGCCGTCCAG
 CAAAGGCAGCTCCCTGAGTTCTGAGTCATCCTGGTACGACTCCCCTGGGCAATGCTGG
 AGAGCTGAGCGAGGCTGAGGGCTCCTCCCTGGCCCCCGCATGCCGTGACCCAGTCTCCA
 10 TGCCAGCTCCCACCTGGCATGCCAAAAGCCTTCAACCAAAGCTTCCCTCCTCCTC
 CCTCCGGAACTGTACAAAGATGCCAACCTGGGAGCCTCTCCCCCTCAGGTATCCGCCT
 TTCTGATGAATAACATGGGCACGCATGCCAGCCTGAGCAACCGTGTCTTTGCTTCCGA
 CATTGATGTGCCCTCCAGAGTGGCACACGGGACCCATCCAGTACAGTTCCCTCACTCT
 CCCCTGTCGGAAGCCAAAGCCTTGTGAGGAACTGCGAAGAAGGACTCCCTCAAAGC
 15 CAGGATGCGACGGATCAGTGACTGGACGGGAAGCCTCTCAAGGAAGAAAAGGAAACTCCA
 GGAGCCGAGGTCCAAGGAGGGCAGTGACTACTTGACAGTCGCTCTGATGGACTGAATAC
 AGATGTGCAGGGATCCTCCAGGCATCTGCTTTCTGTGGTCAGGGGCTCTACTCAGAT
 CCTGTCTCAGAGAAGTGAATCCACACATGCGATTGGCAGCGATCCCCCTCCGGCAGAACAT
 TTATGAGAATTCATGCGAGAGTTGAAATGAGCAGGACCAACACTGAGAACATAGAAAC
 20 ATCTACAGAAACCGCCGAGTCCAGCAGCGAGTCAGCTCTGGAAACAGCTGGATCT
 GCTCTCGAGAACAGGGGGTGGTCCGGAGGGCCGGGTGGCTCTTCTCAAGCCCCT
 GGTCACTGTGCAGAGGAAAGGAAGCTTGAGCTGGTGGCACGAAGGAAATGAAACAGTA
 CTGGGTAACGCTGAAAGGATGCACGCTGCTGTTATGAGACCTATGGAAGAATTCCAT
 GGATCAGAGCAGTCCCCCTGGTGTGCTCTGTTGCAGAACAGCAGTGCAGTCTGT
 25 TCCAGAGCATCCAAAGAAAGAAAATGTGTTCTGCCCTCAGCAACTCCTTGGAGATGTCTA
 CCTTTCCAGGCCACCAGCCAGACAGATCTAGAAAACGGTCAGTGCTACACTCTGC
 TTGTGCATCCCTTTGCAAAGAACAGATGGAAAGAGGACACGCTGCCGTGCTGAAGAA
 CCAGACCAAAACCTGCTCAGAAGATAGACATGGACAGCAAGATGAAGAACAGTGGCAGA
 GCTGCAGCTGTCCGGTGAGCGACCCAAAGAACAGGAAAGCCATAGAGAACCCAGATCCA
 30 GCAATGGGAGCAGAATCTGAGAAATTTCACATGGATCTGTTAGGATGCGCTGCTATCT
 GGCCAGCCTACAAGGTGGGAGTTACCGAACCCAAAGAGTCTCCTTGCAGCCGCCAGCCG
 CCCCTCCAAGCTGGCCCTGGCAGGCTGGCATTTGTCTGTTCCCTTTCCATGCTCT

GGTATGTTCTAGAGATGACTCTGCTCTCCGAAAAGGACACTGTCACTGACCCAGCGAGG
GAGAAACAAGAAGGAAATATTCTCGTAAAAGGGCTGGACACACTGCCAGAAAAGG
CAAGGAGAAGAGACCTTCTATAACTCAGGTCGATGAACCTCTGCATATATATGGTCAAC
AGTGGACGGTGTCCCCGAGACAATGCATGGAAATCCAGACTTATGTTCACTTCAGGA
5 CAATCACGGAGTTACTGTAGGGATCAAGCCAGAGCACAGAGTAGAAGATATTGACTTT
GGCATGCAAGATGAGGCAGTTGGAACCCAGCCATTATGCCCTACAGCTCGAAAATTAGT
AGATGACAATGTTGAGTATTGCATCCCTGCACCATATGAATATATGCAACAACAGGTTA
TGATGAAATAGAAGTCTTCCACTAAATGTTATGATGTGCAGCTCACGAAGACTGGGAG
TGTGTGTGACTTGGGTTGCAGTTACAGCGCAGGTGGATGAGCGTCAGCATCTCAGCCG
10 GATATTTATAAGCGACGTTCTCCGATGCCCTGGCGTATGGGAAGGGCTGAGAAAGGG
CAATGAGATCATGACCTTAAATGGGAAGCTGTCTGATCTGACCTTAAGCAGATGGA
GCCCTGTTCTGAGAACAGCGTCGGACTCACTCTGATTGCCCGCCTCCGGACACAAA
AGCAACCCTGTGTACATCCTGGTCAGACAGTGACCTGTTCTCCAGGGACCAGAACAGTCT
GCTGCCCTCCTAACAGTCCAACTGCTGGAGGAATTCTGGATAACTTAAAAAGAA
15 TACAGCCAATGATTCAAGCAACGTCCTGATATCACAAACAGGTCTGAAAAGGAGTCAGAC
AGATGGCACTCTGGATCAGGTTCCCACAGGGAGAAAATGGAGCAGACATTAGGAGTGC
TGAGCAGATCACTGCAGTGTGCAGGAGTTAACGACAGTCAGGCCAACGGCATGGAAGG
ACCGCGGGAGAACAGGATCAGGATCCTCCCTCCGAGGCCTCTGGCCGCCACCTGTCTGATGCAGA
CCGCCTCCGCAAAGTCATCCAGGAGCTGTGGACACAGAGAACAGTCTACGTGAAGGATT
20 GAGCTGCCCTTTGAATTATACTTGGAGCCACTTCAGAAATGAGACCTTCTAACCAAGA
TGAGATGGAGTCACTTTGGAAAGTTGCCAGAGATGCTTGAGTTCAGAAGGTGTTCT
GGAGACCCCTGGAGGATGGATTTCAGCATCATCTGACTTTAACACCCCTAGAAACCCCTC
ACAGTTAGAAAATTACTGTTCCCTGGAGGCTTTCTTATTACGCGGACCAACTT
TAAACTGTACAGTGGATTCTGTGCTAACCATATCAAAGTACAGAACAGGTTCTGGAGCGAGC
25 TAAAACGTACAAAGCCTCAAGGCTTCTGGATGCCGAACCCACCAAGCAGCATT
CTCCACGCTGGAGTCCTACCTCATCAAGCCGGTCAGAGAGTGCTCAAGTACCGCTGCT
GCTCAAGGAGCTGGTGTCCCTGACGGACCAGGAGAGCGAGGAGCACTACCAACCTGACGGA
AGCACTAAAGGCAATGGAGAAAGTAGCGAGCCACATCAAATGAGATGAGAACAGAGATCTATGA
GGATTATGGGACCGTGTGGACCAGCTAGTAGCTGAGCAGAGCGGAACAGAGAACAGGAGG
30 AACAGAACCTTCGATGGGAGAGCTTCTGATGCACTCTACGGTTCTGGTTGAATCCATT
TCTGTCTCTAGGAAAAGCTAGAAAGGACCTTGAGCTCACAGTATTGTTTAAGAGAGC
CGTCATACTGGTTATAAGAAAATGCAAACGTAAAAAGAAATTGCCCTCGAATTCCCG

GCCTGCACACAACCTACTGACTTGGACCCATTAAATTCCGCTGGTTGATCCCCATCTC
 CGCGCTTCAAGTCAGACTGGGAATCCAGCAGGGACAGAAAATAATTCCATATGGGAACCT
 GATCCATACGAAGTCAGAAATAGAAGGACGCCAGAAACCATCTTCAGTTGTGTTGCAG
 TGACAGTCAAAGCAAAACCAACATTGTTAAGGTGATTGTTCTATTCTGAGGGAAAACCTT
 5 CAGGCGTCACATAAAGTGTGAATTACCACTGGAGAAAACGTGTAAGGATGCCCTGGTACC
 TCTTAAGAACCGAGTTCCCTGTTCGGCCAAATTAGCTTCATCCAGGTCTTAAAAGTCCT
 GAAGAATTCCCTCCAGCAACGAGTGAGCAGCCGGTGGACTGGCAAGGGAACCTGCTGGACTC
 TGACGAGGGCAGCTTGAGCAGCCGGCACCCAGAGCAGCGGCTGCCAACGGCTGAGGGCAG
 GCAGGACTCCAAGAGCACTCTCCCAGGAAATACCCACACCCCGGCTGGCAGATTTC
 10 CGACAATCTCATCAAAGAGAGTGACATCCTGAGCGATGAAGATGATGACCACCGTCAGAC
 TGTGAAGCAGGGCAGCCCTACTAAAGACATCGAAATTCAAGTCCAGAGAGACTGAGGATTTC
 CGAGGACCCAGACGTTCACCCCGAGGCTGAGCAGCAGCCTGGCCGGAGTCGGGTGAGGG
 TCAGAAAGGAGGAGAGCAGCCAAACTGGTCCGGGGGACTTCTGCCCAATTAAACGAAA
 AGCCAACAGCACCAAGAGGGACAGAGGAACCTTGCTCAAGGCGCAGATCCGTACCAAGTC
 15 CCTTGACAGTCAGTCTGAAAATGCCACCATCGACCTAAATTCTGTTCTAGAGCGAGAATT
 CAGTGTCCAGAGTTAACATCTGTTGTCAGTGAGGAGTGTGAAACAGAGAGCCA
 CGGAAAATCATAGTTATGATTCAATCCAGATATGGGTTAAATTCTCATTTACTTTAAA
 CTGGTGGTAAAGTGGAAATTGCAAAAAAAAAAAAAA (SEQ ID NO:1).

The human 33521 sequence (Fig. 1; SEQ ID NO:1) is approximately 5437
 20 nucleotides long. The nucleic acid sequence includes an initiation codon (ATG) and a
 termination codon (TAG) which are underscored above. The region between and inclusive
 of the initiation codon and the termination codon is a methionine-initiated coding sequence
 of about 5106 nucleotides, including the termination codon (nucleotides indicated as
 "coding" of SEQ ID NO:1; SEQ ID NO:3). The coding sequence encodes a 1701 amino acid
 25 protein (SEQ ID NO:2), which is recited as follows:

MGNSDSQYTLQGSKNHSNTITGAKQI~~PCSL~~KIRGVHAKEEKSLHGWHGSNGAGYKS
 RSLARSCLSHFKSNQPYASRLGGPTCKVSRGVAYSTHR~~TNAP~~GKDFQGISA~~F~~STENGFHSV
 GH~~E~~LADNHITSRDCNGHLLNCYGRNESIASTPPGEDRKSPRVLI~~K~~TLGKD~~G~~CLRVEFHNGG
 NPSKVPAEDCSEPVQLLRYSP~~T~~LASETSPVPEARRGSSADSLPSHR~~P~~SPTDSRLRSSKGSSL
 30 SSESSWYDSPWGNAGELSEAEGSFLAPGMPDPSL~~H~~ASFPPGDAKKPFNQSSSLSSLRELYKD
 ANLGSLSPSGIRLSDEYMGTHASLSNRVSFASDIDVPSRVAHGDPIQYSSFTLPCRKPKA~~F~~
 EDTAKKDSLKARMRRISDW~~T~~GSLSRKKRKLQEPRSK~~E~~GSDFDSRSDGLNTDVQGSSQASAF

LWSGGSTQILSQRSESTHAIGSDPLRQNIYENFMRELEMSRTNTENIETSTETAESSSESLS
 SLEQLDLLFEKEQGVVRAGWLFFKPLTVQKERKLELVARRWKQYWVTLKGCTLFFYETY
 GKNNSMDQSSAPRCALFAEDSIVQSVPEHPKKENVFCLSNSFGDVYLFQATSQTDLENWVTAV
 HSACASLFAKKHGKEDTLRLLKNQTKNLLQKIDMDSKMKMAELQLSVSDPKNRKAIENQI
 5 QOWEQNLEKFHMDLFRMRCYLASLQGGELPNPKSLLAAASRPSKLALGRLGILSVSSFHALV
 CSRDDSLRKRTLSLTQRGRNKKGIFSSLKGDLTLARKGKEKRPSITQVDELLHIYGSTVDG
 VPRDNAWEIQTYVHFQDNHGVTVGIKPEHRVEDILTLACKMRQLEPSHYGLQLRKLVDDNVE
 YCIPAPYEMQQQVYDEIEVFPLNVYDVQLTKTGSVCDGFVAVTAQVDERQHLSRIFISDVL
 PDGLAYGEGLRGNEIMTLNGEAVSDLNLKQMEALFSEKSVGLTLIARPPTKATLCTSWSD
 10 SDLFSRDQKSLLPPPQNOSQLLEEFLDNFKKNTANDFSNVPDITTGLKRSQTDGTLQVSHRE
 KMEQTFRSAEQITALCRSFNDSQANGMEPRENQDPPPRPLARHLSADRLRKVIQELVDTE
 KSYVKDLSCLFELYLEPLQNETFLTQDEMESLFGSLPEMLEFQKVFLETLEDGISASSDFNT
 LETPSQFRKLLFSLGGSFLYYADHFKLYSGFCANHIKVQKVLERAKTDKAFLDARNPTK
 QHSSTLESYLIKPVQRVLKYPLLKELVSLTDQESEEHYLTEALKAMEKVASHINEMQKIY
 15 EDYGTVDQLVAEQSGTEKEVTELMSGELLMHSTVSWLNPFLSLGKARKDLELTVFVFKRAV
 ILVYKENCKLKKKLPNSRPAHNSTDLPFKFRWLIPISALQVRLGNPAGTENNISIWEIHT
 KSEIEGRPETIFQLCCSDSESKTNIVKVIIRSILRENFRRIKCELPLEKTCKDRLVPLKNRV
 PVSASKLASSRSILKVLKNSSSNEWTGETKGTL LDSDEGS LSSGTQSSGCPTAEGRQDSKSTS
 PGKYPHPGLADFADNLIKESDILSDEDDHRQTVKQGSPTKDIEIQFQRLRISEDPDVHPEA
 20 EQQPGPESGEGQKGGEQPKLVRGHFCPIKRKANSTKDRGTLKAQIRHQSLDSQSENATID
 LNSVLEREFQSVSLTSVVSEECFYETESHGKS (SEQ ID NO:2).

Example 2: Tissue Distribution of 33521 mRNA by TaqMan Analysis

Endogenous human 33521 gene expression was determined using the Perkin-Elmer/ABI 7700 Sequence Detection System which employs TaqMan technology. Briefly, 25 TaqMan technology relies on standard RT-PCR with the addition of a third gene-specific oligonucleotide (referred to as a probe) which has a fluorescent dye coupled to its 5' end (typically 6-FAM) and a quenching dye at the 3' end (typically TAMRA). When the fluorescently tagged oligonucleotide is intact, the fluorescent signal from the 5' dye is quenched. As PCR proceeds, the 5' to 3' nucleolytic activity of Taq polymerase digests the labeled primer, producing a free nucleotide labeled with 6-FAM, which is now detected as a fluorescent signal. The PCR cycle where fluorescence is first released and detected is 30

directly proportional to the starting amount of the gene of interest in the test sample, thus providing a quantitative measure of the initial template concentration. Samples can be internally controlled by the addition of a second set of primers/probe specific for a housekeeping gene such as GAPDH which has been labeled with a different fluorophore on 5 the 5' end (typically VIC).

To determine the level of 33521 in various human tissues a primer/probe set was designed. Total RNA was prepared from a series of human tissues using an RNeasy kit from Qiagen. First strand cDNA was prepared from 1 µg total RNA using an oligo-dT primer and Superscript II reverse transcriptase (Gibco/BRL). cDNA obtained from approximately 50 ng 10 total RNA was used per TaqMan reaction. Tissues tested include the human tissues and several cell lines shown in Table 1. 33521 mRNA was detected in glioblastoma cells. As compared to their normal tissue counterparts, elevated levels of expression were observed in breast, colon, and lung tumors.

15 Table 1. Expression of 33521 in various tissues

Tissue	Expression
Glioblastoma	21.33
Glioblastoma	22.78
Glioblastoma	48.17
Glioblastoma	59.71
Brain Normal	11.67
Placenta Normal	5.58
Skin Normal	9.68
Adipose Normal	1.18
Breast Normal	3.89
Breast Tumor	100.78
Breast Tumor	5.62
Breast Tumor	23.34
Colon Normal	1.13
Colon Tumor	16.91
Colon Tumor	6.73
Colon Tumor	19.56
Liver Normal	1.20
Colon Metastasis	12.21
Colon Metastasis	9.99
Lung Normal	1.00
Lung Tumor	5.98

Lung Tumor	11.24
Lung Tumor	4.08

Example 3: Tissue Distribution of 33521 mRNA by Northern Analysis

Northern blot hybridizations with various RNA samples can be performed under standard conditions and washed under stringent conditions, i.e., 0.2xSSC at 65°C. A DNA probe corresponding to all or a portion of the 33521 cDNA (SEQ ID NO:1) can be used. The DNA was radioactively labeled with 32 P-dCTP using the Prime-It Kit (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing mRNA from mouse hematopoietic and endocrine tissues, and cancer cell lines (Clontech, Palo Alto, CA) can be probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

Example 4: Recombinant Expression of 33521 in Bacterial Cells

In this example, 33521 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, 33521 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-33521 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

Example 5: Expression of Recombinant 33521 Protein in COS Cells

To express the 33521 gene in COS cells (e.g., COS-7 cells, CV-1 origin SV40 cells; Gluzman (1981) *Cell* 23:175-182), the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire 33521 protein and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-

frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the 33521 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately 5 twenty nucleotides of the 33521 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 33521 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen 10 are different so that the 33521 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5 α , SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from 15 transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the 33521-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. (1989) 20 *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. The expression of the 33521 polypeptide is detected by radiolabelling (35 S-methionine or 35 S-cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. (1988) 25 *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with 35 S-methionine (or 35 S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% 30 DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the 33521 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the 33521 polypeptide is detected by radiolabelling and immunoprecipitation using a 33521 specific monoclonal antibody.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

10

WHAT IS CLAIMED IS:

- 1 1. An isolated nucleic acid molecule selected from the group consisting of:
 - 2 a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID
 - 3 NO:3, or a complement thereof; and
 - 4 b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid
 - 5 sequence of SEQ ID NO:2.
- 1 2. The nucleic acid molecule of claim 1, further comprising a vector nucleic acid
- 2 sequence.
- 1 3. The nucleic acid molecule of claim 1, further comprising a nucleic acid sequence
- 2 encoding a heterologous polypeptide.
- 1 4. A host cell which contains the nucleic acid molecule of claim 1.
- 1 5. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2.
- 1 6. The polypeptide of claim 5, further comprising heterologous amino acid
- 2 sequences.
- 1 7. An antibody or antigen-binding fragment thereof that selectively binds to the
- 2 polypeptide of claim 5.
- 1 8. A method for producing a polypeptide comprising the amino acid
- 2 sequence of SEQ ID NO:2, the method comprising culturing the host cell of claim 4
- 3 under conditions in which the nucleic acid molecule is expressed.
- 1 9. A method for detecting the presence of the polypeptide of claim 5 in a sample, the
- 2 method comprising:
 - 3 a) contacting the sample with an antibody that selectively binds to the polypeptide;
 - 4 and

- 5 b) determining whether the compound binds to the polypeptide in the sample.
- 1 10. A kit comprising a compound that selectively binds to the polypeptide of claim 5
2 and instructions for use.
- 1 11. A method for detecting the presence of the nucleic acid molecule of claim 1 in a
2 sample, the method comprising:
 - 3 a) contacting the sample with a nucleic acid probe or primer that selectively
4 hybridizes to the nucleic acid molecule; and
 - 5 b) determining whether the nucleic acid probe or primer binds to a nucleic acid in the
6 sample.
- 1 12. The method of claim 11, wherein the sample comprises mRNA molecules and is
2 contacted with a nucleic acid probe.
- 1 13. A kit comprising a nucleic acid probe or primer that selectively hybridizes to the
2 nucleic acid molecule of claim 1 and instructions for use.
- 1 14. A method for identifying a compound that binds to the polypeptide of claim 5, the
2 method comprising:
 - 3 a) contacting the polypeptide or a cell expressing the polypeptide with a test
4 compound; and
 - 5 b) determining whether the polypeptide binds to the test compound.
- 1 15. A method for modulating the activity of the polypeptide of claim 5, the method
2 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound
3 that binds to the polypeptide in a sufficient concentration to modulate the activity of the
4 polypeptide.
- 1 16. A method of inhibiting aberrant activity of a 33521-expressing cell, comprising
2 contacting the cell with a compound that modulates the activity or expression of the

3 polypeptide of claim 5, in an amount that is effective to reduce or inhibit the aberrant activity
4 of the cell.

1 17. The method of claim 16, wherein the compound is selected from the group
2 consisting of a peptide, a phosphopeptide, a small organic molecule, and an antibody.

1 18. The method of claim 16, wherein the 33521-expressing cell is a cancer cell.

1 19. A method of treating or preventing a disorder characterized by aberrant activity of
2 a 33521-expressing cell, in a subject, the method comprising administering to the subject an
3 effective amount of a compound that modulates the activity or expression of the nucleic acid
4 molecule of claim 1, such that the aberrant activity of the-expressing cell is reduced or
5 inhibited.

1 20. The method of claim 19, wherein the 33521-expressing cell is a cancer cell.

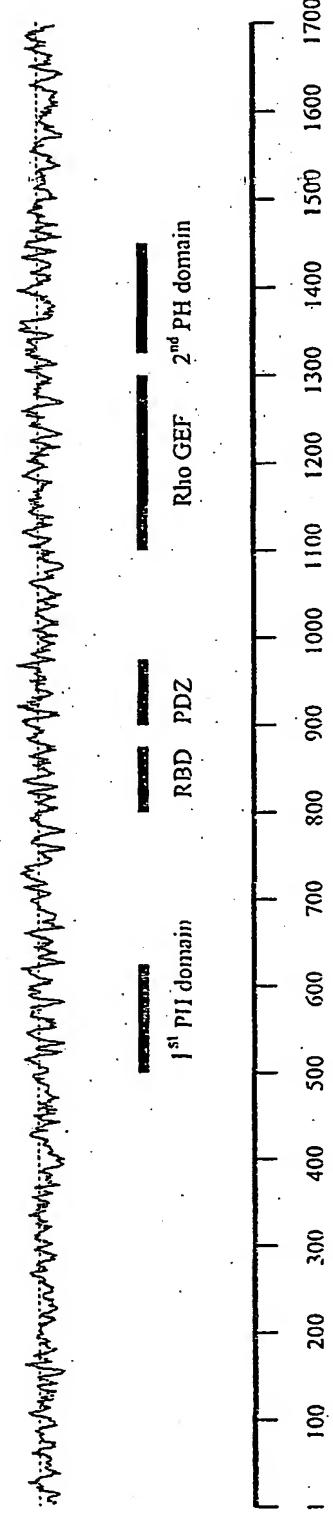


FIG. 1

PH: domain 1 of 2, from 507 to 620: score 65.9; E = 4.1e-17
*->viikeGwLlkks.....kswkkRyfvLfnnvLlyykds
v + GwL+ k + +++++ + +++wk++++L++ +Ll+y+
33521 507 VRRAGWLFFKPlvtvqkerklelvarRKWKQYWVTLKGCTLLFYETY 553

k.....kkpkgsipLsgcqvek.pd....kncFeirt.dr.tlllqae
++++ ++++ p+ + +++++v p++++++n+F+++++ +++++l+qa+
33521 554 GknsmdqssAPRCALFAEDSIVQSvPEhpkkENVFCLSNsFGdVYLFQAT 603

seeerkeWvkaiqsair<-* (SEQ ID NO:4)
s+ +++ Wv+a +sa++
33521 504 SQTDLENWVTAVHSACA 620

FIG. 2A

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RBD: domain 1 of 1, from 810 to 873: score -3.0, E = 2.3
*-->ktirvhLPnnqrsVevRpGmtvrDaLakalkkRGLnpsacvVrrsg
+ vh +n ++V +p +v+D+L a+k+R+L ps +
33521 810 IQTYVHFQDNHGVTVGIKPEHRVEDILTLACKMRQLEPSHYGLQ--- 853
dpqeGekkpLdldtdissLpgPeElvvEnl<-* (SEQ ID NO:5)
L+ d + + +p+P +E
33521 854 -----LRKLVDDNVEYCIPAP----YEYM 873

FIG. 2B

PDZ: domain 1 of 1, from 890 to 975: score 33.5, E = 4.8e-06
*->eitlekevkrgglGfsikgg sdk....givvsevlpGsgaAeagGr
+++l k + +Gf + + d++++ + i++stvlp+ g A G
33521 890 DVQLTKTGSVCDFGFAVTAQVDERqhl sRIFI SDVLPD-GLAYGEG- 934

LkeGDvIls vNGqdvenmsheravilaikgsgg.evtLtv lRd<-*(SEQ ID NO:6)
L++G I+ NG+ v++ ++++++a+ + ++ +tL +++
33521 935 LRKGNEIMTLNGEAVSDL-DLKQMEALFSEKSvGLTLIARPP 975

FIG. 2C

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RhoGEF: domain 1 of 1, from 1103 to 1292: score 147.0, E = 3.3e-40
*->vlkElleTEkkYvrdLeildnvymkpLreaaisskpvltpddietif
v+ El+ TEk Yv+dL l y++pL+++ +lt+d++e++F
33521 1103 VIQELVDTEKSYVKDLSCLFELYLEPLQNET-----FLTQDEMESLF 1144

sNiediyefhreFLkssLearisssqfedlDe....kkiepsaprlGd1F
+ +++ +ef++ FL Let is+s d + +++ + 1G F
33521 1145 GSLPEMULEFQKVFL-E-TLEDGISAS--SDFNTletpSQFRKLLFSLGGSF 1191

iklikepflqvYgeYcsnkpyAqelleklrqaaasnpgFaefldeveassnt
l + ++ +++Y +c+n+ +q++le+ a++ +E+ fld + + +
33521 1192 LYY-ADHFKLYSGFCANHIKVQKVLER---AKTDKAFAFLDARNPTKQH 1237

gAkddavkltlqsLLLkPvqRilrYpLLLkeLlkhtpegedqpdredLkk
tL+s+L+kPvqR+l+YpLLLkeL+++t+ +++++ L +
33521 1238 S-----STLESYLIKPVQRVLKYPLLKELVSLTDQ--ESEEHYHLTE 1278

aldllqdlaksiNe<-*(SEQ ID NO:7)
al++++ +a++iNe
33521 1279 ALKAMEKVASHINE 1292

FIG. 2D

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PH: domain 2 of 2, from 1353 to 1455: score 4.3, E = 8.7

*->fvLfnnvLlyykdsk.....kkpkgsipLsg

fv+++ v+l yk+ + +++ ++++++ +++++ +++k + . ips

33521 1353 FVFKRAVILVYKENCklkkklpsnsrpahnstdldpFKFRWLIPISA 1399

cqvek.pd.....kncFeirt.dr.....tllqaeseeerkeWvka

+qv+ + +++++ +e+++ + +++++ + l++++++ vk

33521 1400 LQVRLgNPagtenNSIWELIhtKSeiegrpetIFQLCCSDSESKTNIVKV 1449

iqsair<--*(SEQ ID NO:8)

i+s+r

33521 1450 IRSILR 1455

FIG. 2E

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PH_update: domain 1 of 2, from 507 to 622: score 63.3, E = 5.2e-15
*->vikeGwLlkks.....k.swkkryfvLfngvLllyyksk
v + GwL k + ++ ++ +++wk+++++L++ +Ll+y+
33521 507 VRRAGWLFFKPlvtvqkerklelvaRrKWKQYWVTLKGCTLLFYETY 553

.....kpkgsipLsgcsvre.p.....cFeivt.dr.tlllqAe
++++ ++++++p+ ++ ++ +v+ p+++++++F+++++ ++++++l+qA
33521 554 gknsmdqssAPRCALFAEDSIVQSvPehpkkenVFCLSNsFGdVYLFQAT 603

seeereewvealqsaiaka<-* (SEQ ID NO:9)
st.++e Wv+a +sata++
33521 604 SQTDLENWVTAVHSACASL 622

FIG. 3A

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RBD_5: domain 1 of 1, from 810 to 881: score 68.6, E = 1.3e-16
*->ktcrvhLPdnqrtvVkvRPGktvrDaLakaLkkRgLnpeacvVrlrg
++++vh++dn +++V ++P+++v+D+L +a+k+R+L+p++++++lr+
33521 810 IQTYVHFQDNHGVTVGIKPEHRVEDILTLACKMRQLEPSHYGLQLRK 856

dpqeGekkpldlnqdissLaggElvveel<-* (SEQ ID NO:10)
++ ++ + ++ ++++q +v++e+
33521 857 L----VDDNVEYCIPAPYEMQQQVYDEI 881

FIG. 3B

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pdz_new: domain 1 of 1, from 900 to 976: score 39.3, E = 8.9e-08
*->gglGfsivg.....gifvssvvpGspAakaGrkslglLkvGDv
+Gf ++ + +++++ + if+s+v+p++ A+ G L++G +
33521 900 CDFGFAVTAqvderqhlRsRIFISDVLPDGLAYGEG-----LRKGNE 940

IleVNGetsvegltheeavdlkkaggggvGekvtLtv1Rgg<-* (SEQ ID NO:11)
I+ NG + v++l+ + +l + + +tL+ +++.
33521 941 IMTLNG-EAVSDLQKMEALFSEKSV-----GLTLIARPPD 976

FIG. 3C

10/11

RhoGEF_3: domain 1 of 1, from 1103 to 1292: score 217.7, E = 1.8e-61
*->vlkELLqTERnYVrdLkilvevflkpLkkeakssllplspdevktl
v++EL++TE++YV+dL +l e +l+pL++e +l++de+++l
33521 1103 VIQELVDTEKSYVKDLSCLFELYLEPLQNET-----FLTQDEMESL 1143

FgpNieeiyefHrrFLdeLeervéewllskdl.Ksernsviedsgerigd
Fg ++e +ef++ FL++Le+ ++ + d++ e+ s++++ +g
33521 1144 FG-SLPEMLEFQKVFLLETLEDGISASS---DFnTLETPSQFRKLLFSLGG 1189

vFlklfsaeffkiYseYcsnhpdalellkkLmkkknpaFqkf1keies
F1 + +++fk+Ys +c+nh + +++l+ +k+++aF++f1+ +
33521 1190 SFLYY---ADHFKLYSGFCANHIKVQKVLER---AKTDKAFAFLDARNP 1233

kpnccrsksearltLeslLikPvQRltkYpLLLkelkhtppdhedredLk
+*** tLes+LikPvQR++kYpLLLkel t+ ++e++ +L+
33521 1234 TKQHS-----STLESYLIKPVQRVLKYPLLKELVSLTDQESEEHYHLT 1277

kAleaikeLasqvNe<-* (SEQ ID NO:12)
+Al+a+++as++Ne
33521 1278 EALKAMEKVASHINE 1292

FIG. 3D

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PH_update: domain 2 of 2, from 1326 to 1457: score 17.7, E = 0.28

...vikeGwLlkks.....k.swkkryfvlEngviliyyksk.....

++++ ++++++ + + ++ ++ fv+++ v+l yk+ + +++

33521 1326 LLMHSTVSWLNpfslgkaRkDLELTVFVFKRAVILVYKENcklk 1372

.....pkgsipLsgcsvre.p.....cFeivt.d

++++++ ++++++ ++ k + ips ++vr + + + ++++ +e+++++

33521 1373 lpsnsrpahnstdldpfKFRWLIPISALQVRLgNpagtennsIWELIhtK 1422

r.....tllqAeseeereewvealqsaika<-* (SEQ ID NO:13)

+ ++++++ + l+++++e+++ vt ++s +++

33521 1423 SeiegrpetIFQLCCSDSESKTNIVKVIRSILREN 1457

FIG. 3E

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GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
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WO 02/026817 A3

(54) Title: 33521, A HUMAN GUANINE NUCLEOTIDE EXCHANGE FAMILY MEMBER AND USES THEREOF

(57) Abstract: The invention provides isolated nucleic acids molecules, designated 33521 nucleic acid molecules, which encode novel Rho GEF members. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing 33521 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a 33521 gene has been introduced or disrupted. The invention still further provides isolated 33521 proteins, fusion proteins, antigenic peptides and anti-33521 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

INTERNATIONAL SEARCH REPORT

In International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C12N15/12 C07K14/47 C07K16/18 C12N5/10 G01N33/50
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols).
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 40607 A (CHIRON CORPORATION (US); DUHL DAVID (US)) 13 July 2000 (2000-07-13) SEQ ID NO:6,8-13 the whole document ---	1-20
A	HOSHINO M. ET AL.: "Identification of the stef gene that encodes a novel guanine nucleotide exchange factor specific for Rac1" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 25, 18 June 1999 (1999-06-18), pages 17837-17844, XP002141545 ISSN: 0021-9258 the whole document ---	1-20 -/-

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Patent family members are listed in annex.

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- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

19 July 2002

Date of mailing of the international search report

07/08/2002

Name and mailing address of the ISA

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Authorized officer

Macchia, G

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/29962

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHIU C.-Y. ET AL.: "Cloning and characterization of T-cell lymphoma invasion and metastasis 2 (TIAM2), a novel guanine nucleotide exchange factor related to TIAM1" GENOMICS, vol. 61, no. 1, 1 October 1999 (1999-10-01), pages 66-73, XP000864676 ISSN: 0888-7543 the whole document ---	1-20
A	CHIU C.-Y. ET AL.: "Erratum: Cloning and characterization of T-cell lymphoma invasion and metastasis 2 (TIAM2), a novel guanine nucleotide exchange factor related to TIAM1" GENOMICS, vol. 63, no. 3, 2000, page 439 XP000864677 ISSN: 0888-7543 the whole document ---	
X	WO 97 46680 A (CHIRON CORPORATION (US)); DUHL DAVID (US)) 11 December 1997 (1997-12-11) SEQ ID NO:6,7 the whole document ---	7,9-20
X	DATABASE EMBL 'Online' 1 February 2000 (2000-02-01) "Mus musculus T-cell lymphoma invasion and metastasis 2 (TIAM2) mRNA, partial cds." Database accession no. AF121102 XP002206679 the whole document ---	7
X	DATABASE EMBL 'Online' 25 October 1999 (1999-10-25) "Homo sapiens SIF and TIAM1-like exchange factor mRNA, partial cds." Database accession no. AF195656 XP002206680 the whole document ---	7
X	DATABASE EMBL 'Online' 18 February 2000 (2000-02-18) "Homo sapiens mRNA; cDNA DKFZp43L1072; partial cds. Strong similarity to mouse Stef." Database accession no. AL122086 XP002206681 the whole document ---	7

INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 15-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: 10, 15-17, 19 all in part because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.2

Claims Nos.: 10, 15-17, 19 all in part

Present claims 10, 15-17 and 19 contain a reference to compounds defined by reference to a desirable characteristic or property, namely their binding to the polypeptide of present invention and/or their ability to modulate the activity or expression of said polypeptide.

The claims cover all compounds having this characteristic or property, whereas only a very limited number of such compounds could be derived from present application, namely antibodies against the polypeptide of present invention and complementary polynucleotides (antisenses, ribozymes) against the polynucleotide encoding said polypeptide.

In the present case, the claims so lack support (Article 6 PCT), and the application so lacks disclosure (Article 5 PCT), that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define a compound by reference to a result to be achieved.

Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to antibodies against the polypeptide of present invention and complementary polynucleotides (antisenses, ribozymes) against the polynucleotide encoding said polypeptide.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/29962

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WO 0040607	A 13-07-2000	AU	2409700 A	24-07-2000
		EP	1144448 A2	17-10-2001
		WO	0040607 A2	13-07-2000
WO 9746680	A 11-12-1997	AU	3230797 A	05-01-1998
		EP	0938553 A1	01-09-1999
		JP	2000513571 T	17-10-2000
		WO	9746680 A1	11-12-1997
		US	6300484 B1	09-10-2001